EXHIBIT E
Part 1 of 2

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United States Patent [19]

Lin

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[54] **PRODUCTION OF ERTHROPOIETIN**

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[58] **Field of Search** 424/439; 435/69.1, 435/69.2, 69.3, 69.6, 71.1, 71.2, 172.1, 172.3; 436/8; 514/8, 778, 970, 12; 530/351, 361, 395, 350

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,033,753	5/1962	White et al.	530/395
3,865,801	2/1975	Chiba et al.	530/397
4,237,224	12/1980	Cohen et al.	435/69.1
4,254,095	3/1981	Fisher et al.	436/513
4,264,731	4/1981	Shine	435/91.41
4,273,875	6/1981	Manis	435/91.4
4,293,652	10/1981	Cohen	435/91.1
4,303,650	12/1981	Takezawa et al.	424/177
4,338,397	7/1982	Gilbert et al.	435/69.1
4,358,535	11/1982	Falkow et al.	435/5
4,377,513	3/1983	Sugimoto et al.	530/395
4,394,443	7/1983	Weissman et al.	435/6
4,397,840	8/1983	Takezawa et al.	424/99
4,399,216	8/1983	Axel et al.	435/6
4,411,994	10/1983	Gilbert et al.	435/69.7
4,442,205	4/1984	Hamer et al.	435/69.3
4,465,624	8/1984	Chiba et al.	530/395
4,468,464	8/1984	Cohen et al.	435/320.1
4,503,151	3/1985	Paddock	435/69.1
4,517,294	5/1985	Bock et al.	435/70
4,558,005	12/1985	Goldwasser et al.	435/7.92
4,558,006	12/1985	Egrie	435/7.94
4,568,488	2/1986	Lee-Huang	530/397
4,667,016	5/1987	Lai et al.	530/397
4,677,195	6/1987	Hewick et al.	514/8
4,695,542	9/1987	Yokata et al.	435/69.4
4,703,008	10/1987	Lin	435/360
4,710,473	12/1987	Morris	435/320.1
4,757,006	7/1988	Toole et al.	435/69.6
4,806,524	2/1989	Kawaguchi et al.	514/8

FOREIGN PATENT DOCUMENTS

0070685	1/1983	European Pat. Off. .
0070687	1/1983	European Pat. Off. .
0077670	4/1983	European Pat. Off. .
0093619	11/1983	European Pat. Off. .
0123294	4/1984	European Pat. Off. .
0116446	8/1984	European Pat. Off. .
0117058	8/1984	European Pat. Off. .
0117059	8/1984	European Pat. Off. .
0117060	8/1984	European Pat. Off. .
0136490	4/1995	European Pat. Off. .
33 16 297 A1	11/1983	Germany .
33 48 289 C2	11/1983	Germany .
2085887	5/1982	United Kingdom .
83/04053	11/1983	WIPO .
85/01961	5/1985	WIPO .
85/03079	7/1985	WIPO .
85/04419	10/1985	WIPO .
86/03520	6/1986	WIPO .

OTHER PUBLICATIONS

U.S. Ser. No. 483,451, Alton et al.
U.S. Ser. No. 487,751, Bitter.
U.S. Ser. No. 693,258, Fritsch et al.
Abraham et al., "Nucleotide Sequence of a Bovine Clone Encoding the Agiogenic Protein, Basic Fibroblast Growth Factor," *Science*, 233, 545–548 (Aug. 1, 1986).
Adamson, "The Polycythemias: Diagnosis and Treatment," *Hosp. Practice*, 18(12), 49–57 (Dec. 1983).
Aebi et al. "Sequence Requirements for Splicing of Higher Eukaryotic Nuclear Pre–mRNA," *Cell*, 47, 555–565 (Nov. 21, 1986).
Agarwal et al., "A General Method for Detection and Characterization of an mRNA using an Oligonucleotide Probe," *J. Biol. Chem.*, 256, 1023–1028 (Jan. 25, 1981).

(List continued on next page.)

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[57] **ABSTRACT**

Disclosed are novel polypeptides possessing part or all of the primary structural conformation and one or more of the biological properties of mammalian erythropoietin ("EPO") which are characterized in preferred forms by being the product of procaryotic or eucaryotic host expression of an exogenous DNA sequence. Illustratively, genomic DNA, cDNA and manufactured DNA sequences coding for part or all of the sequence of amino acid residues of EPO or for analogs thereof are incorporated into autonomously replicating plasmid or viral vectors employed to transform or transfect suitable procaryotic or eucaryotic host cells such as bacteria, yeast or vertebrate cells in culture. Upon isolation from culture media or cellular lysates or fragments, products of expression of the DNA sequences display, e.g., the immunological properties and in vitro and in vivo biological activities of EPO of human or monkey species origins. Disclosed also are chemically synthesized polypeptides sharing the biochemical and immunological properties of EPO. Also disclosed are improved methods for the detection of specific single stranded polynucleotides in a heterologous cellular or viral sample prepared from, e.g., DNA present in a plasmid or viral-borne cDNA or genomic DNA "library".

2 Claims, 27 Drawing Sheets

OTHER PUBLICATIONS

Anderson et al., "Isolation of a genomic clone for bovine pancreatic trypsin inhibitor by using a unique–sequence synthetic DNA probe," *P.N.A.S.* (*USA*), 80, 6838–6842 (Nov. 1983).

Antonsson et al., "Posttranslational Modifications of Fibromodulin," *J. Biol. Chem.*, 266(25), 16859–16861 (1991).

Baciu et al., "Erythropoietin Interaction with the Mature Red Cell Membrane," *Ann. N.Y. Acad. Sci.*, 414, 66–72 (1983).

Baron et al., "Antibodies against the Chemically Synthesized Genome–Linked Protein of Poliovirus React with Native Virus–Specific Proteins," *Cell*, 28, 395–404 (Feb. 1982).

Beaucage et al., "Deoxynucleoside Phosphoramidites—A new Class of Key Intermediates for Deoxypolynucleotide Synthesis," *Tetrahedron Letters*, 22(20), 1859–1862 (1981).

Benedum et al., "The primary structure of bovine chromogranin A: a representative of a class of acidic secretory proteins common to a variety of peptidergic cells," *EMBO J.* 5(7), 1495–1502 (1986).

Bennetzen et al., "Codon Selection in Yeast," *J. Biol. Chem.*, 257(6), 3026–3031 (Mar. 25, 1982).

Bentley et al., "Human immunoglobulin variable region genes–DNA sequences of two V_k genes and a pseudogene," *Nature* 288, 730–733 (Dec. 1980).

Benton et al., "Screening λgt Recombinant Clones by Hybridization to single Plaques in situ," *Science* 196, 180–182 (Apr. 8, 1977).

Berzofsky et al., "Topographic Antigenic Determinants Recognized by Monoclonal Antibodies to Sperm Whale Myoglobin," *J. Biol. Chem.* 257(6), 3189–3198 (Mar. 25, 1982).

Berzofsky et al., "Properties of Monoclonal Antibodies Specific for Determinants of a Protein Antigen, Myoglobin," *J. Biol. Chem.* 255(23), 11188–11191 (Dec. 10, 1980).

Betsholtz et al., "cDNA sequence and chromosomal localization of human platelet–derived gtowth factor A—chain and its expression in tumour cell lines," *Nature* 320, 695–699 (Apr. 24, 1986).

Billat et al., "In Vitro and In Vivo Regulation of Hepatic Erythropoiesis by Erythropoietin and Glucocorticoids in the Rat Fetus," *Exp. Hematol.*, 10(1), 133–140 (1982).

Blattner et al., "Charon Phages: Safer Derivatives of Bacteriophage Lambda for DNA Cloning," *Science*, 196, 161–169 (Apr. 8, 1977).

Bos et al., "Eukaryotic Expression of Cloned cDNA Coding for Influenza Viral Glycoproteins Using an SV40 Vector: Use of Recombinant DNA Mutants to Study Struture–Function Relationships[1]," Proc. Symp. Mol. Biol. Negat., Strand Viruses Meeting, pp. 125–130, Compans et al., eds., Acad. Press (1984).

Bray et al., "Human cDNA clones for four species of Gα–signal transduction protein," *P.N.A.S.*. (*USA*), 83, 8893–8897 (Dec. 1986).

Broome et al., "Immunological screening method to detect specific translation products," *P.N.A.S.* (*USA*), 75(6), 2746–2749 (Jun 1978).

Brown, et al., "Erythropoietin: Gene Cloning, Protein Structure, and Biological Properties," Cold Spring Harbor Symposia on Quantitative Biology, L1, 693–702 (1986).

Canaani et al., "Regulated expression of human interferon β1 gene after transduction into cultured mouse and rabbit cells," *P.N.A.S.* (*USA*), 79, 5166–5170 (Sep. 1982).

Chan et al., "Construction and selection of recombinant plasmids containing full–length complementary DNAs corresponding to rat insulins I and II ," *P.N.A.S.* (*USA*), 76(10), 5036–5040 (Oct. 1979).

Chia et al., "The construction of cosmid libraries of eukaryotic DNA using the Homer series of vectors," *Nucleic Acids Res.* 10(8), 2503–2520 (1982).

Chiba et al., "Stabilization of Urinary Erythropoietin," *Biochem. and Biophys. Res. Commun.*, 47(6), 1372–1377 (1972).

Chirgwin et al., "Isolation of Biologically Active Ribonucleic Acid from Sources Enriched in Ribonuclease," *Biochemistry*, 18(24), 5294–5299 (1979).

Chisholm, "On the Trail of the Magic Bullet: Monoclonal antibodies promise perfectly targeted chemicals," *High Technology*, vol. 2(1), 57–63 (Jan. 1983).

Chomczynski et al., "Alkaline Transfer of DNA to Plastic Membrane," *Biochem. Biophys. Res. Commun.*, 122(1), 340–44 (Jul. 18, 1984).

Choo et al., "Molecular cloning of the gene for human anti–haemophilic factor IX," *Nature*, 299, 178–180 (Sep. 9, 1982).

Choppin et al., "Characterization of Erythropoietin Produced by IW32 Murine Erythroleukemia Cells," *Blood*, 64(2), 341–347 (Aug. 1984).

Chou et al., "Prediction of the Secondary Structure of Proteins from their Amino Acid Sequence," *Advances in Enzymology*, 47, 45–47 (1978).

Chou et al., "Empirical Predictions of Protein Conformation," *Ann. Rev. Biochem.*, 47, 251–77 (1978).

Chou et al., "Prediction of Protein Conformation," *Biochem.*, 13(2), 222–245 (1974).

Christman et al., "Amplification of expression of hepatitis B surface antigen in 3T3 cells cotransfected with a dominant–acting gene and cloned viral DNA," *P.N.A.S.* 79, 1815–1819 (Mar. 1982).

Claus–Walker et al., "Spinal Cord Injury and Serum Erythropoietin," *Arch. Phys. Med. Rehabil.*, 65, 370–375 (Jul. 1984).

Colby et al., "Immunological Differentiation Between *E. coli* and CHO Cell–Derived Recombinant and Natural Human β–Interferons[1]," *J. Immunol.*, 133(6), 3091–3095 (1984).

Collen et al., "Biological Properties of Human Tissue–Type Plasminogen Activator Obtained by Expression of Recombinant DNA in Mammalian Cells," *J. of Pharmacology and Exp. Therapeutics*, 231(1), 146–152 (1984).

Colman , "Cells that secrete foreign proteins,"*TIBS*, 435–437 (Dec. 1982).

Comb et al., "Primary structure of the human Met– and Leu–enkephalin precursor and its mRNA," *Nature*, 295, 663–666 (Feb. 25, 1982).

Congote, "Regulation of Fetal Liver Erythropoiesis," *J. of Steroid Biochemistry*, 3, 423–428 (1977).

Congote, "Extraction from Fetal Bovine Serum of Erythrotropin, an Erythroid Cell–Stimulating Factor," *Anal. Biochem.*, 140, 428–433 (1984).

Congote, "Isolation of Two Biologically Active Peptides, Erythrotropin I and Erythrotropin II from Fetal Calf Intestine," *Biochem. Biophys. Res. Comm.*, 115(2), 477–483 (Sep 15, 1983).

Congote et al., "The Erythrotropins, New Erythroid Cell Stimulating Factors Extracted From Human and Bovine Fetal Tissues," Abstract 364, Proceedings 7th International Congress of Endocrinology (Quebec City, Quebec, Jul. 1–7, 1984).

Contrera et al., "Extraction of erythropoietin from Kidneys of Hypoxic and Phenylhydrazine–treated rats," *Blood*, 25(5), 809–816 (May 1965).

Costantini et al., "Introduction of a Rabbit Betaglobin Gene into the Mouse Germ Line," *Nature*, 294, 92–94 (Nov. 5, 1981).

Costantini et al., "Gene Transfer into the Mouse Germ–Line," *J. Cell Physiol. Supp.* 1, 219–226 (1982).

Cotes et al., "Changes in serum immunoreactive erythropoietin during the menstrual cycle and normal pregnancy," *Brit. J. Obstet. Gynaecol.*, 90, 304–311 (Apr. 1983).

Cotes et al., "Bio–Assay of Erythropoietin in Mice made Polycythaemic by Exposure to Air at a Reduced Pressure," *Nature*, 191, 1065–1067 (Sep. 9, 1961).

Dainiak et al., "Mechanisms of Abnormal Erythropoiesis in Malignancy," *Cancer*, 51(6), 1101–1106 (1983).

Das et al., "Use of synthetic oligonucleotide probes complementary to genes for human HLA–DRα and β as extension primers for the isolation of 5'–specific genomic clones," *P.N.A.S.* (*USA*), 80, 1531–1535 (Mar. 1983).

Davis et al., "A Manual for Genetic Engineering, Advanced Bacterial Genetics", Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1983), pp. 55–58 & 174–176.

Davis et al., "Active Influenza Virus Neuraminidase is Expressed in Monkey Cells from cDNA Cloned in Simian Virus 40 Vectors," *Proc. Nat'l. Acad. Sci.* (*USA*), 80, 3976–3980 (1983).

Derynck et al., "Human transforming growth factor–β complementary DNA sequence and expression in normal and transformed cells," *Nature*, 316, 701–705 (Aug. 22, 1985).

Derynck et al., "Human Transforming Growth Factor–α: Precursor Structure and Expression in *E. coli*," *Cell*, 38, 287–297 (Aug. 1984).

Dessypris et al., "Effect of pure erythropoietin on DNA–synthesis by human marrow day 15 erythroid burst forming units in short–term liquid culture," *Brit. J. Haematol.*, 56, 295–306 (1984).

Devos et al., "Purification of Recombinant Glycosylated Human Gamma Interferon Expressed in Transformed Chinese Hamster Ovary Cells," *J. Interferon Research*, 4,461–468 (1984).

Docherty et al., "Sequence of human tissue inhibitor of metalloproteinases and its identity to erythroid–potentiating activity," *Nature*, 318, 66–69 (Nov. 7, 1985).

Dordal et al., "Function and Composition of the Carbohydrate Portion of Human Urinary Erythropoietin," *Experimental Hematology*, 10, Supp. 11, p. 133, Abstract No. 222 (1982).

Dordal et al., "The Role of Carbohydrate in Erythropoietin Action," *Endocrinology*, 116(6), 2293–2299 (1985).

Dreesman et al., "Antibody to hepatitis B surface antigen after a single inoculation of uncoupled synthetic HBsAG peptides," *Nature*, 295, 158–160 (Jan. 14, 1982).

Dunn et al., "Use of a computer model in the understanding of erythropoietic control mechanisms," *Chemical Abstracts*, 91, 190417r (1979).

Dunn, "Current Concepts in Erythropoiesis", John Wiley & Sons, Chichester, England, 1983.

Dunn et al., "Serum erythropoietin titers during prolonged bedrest; relevance to the "anaemia" of space flight," *Eur. J. Appln. Physiol.*, 52, 178–182 (1984).

Dunn et al., "Erythropoietin Bioassays Using Fetal Mouse Liver Cells: Validations and Technical Improvements," *Exp. Hematol.*, 11(7), 590–600 (Aug. 1983).

Edman et al., "A Protein Sequentator," *Eur. J. Biochem.* 2, 80–91 (1967).

Emmanouel et al., "Metabolism of pure human erythropoietin in the rat," *Am. J. Physiol.*, 247 (1 Pt 2), F 168–76 (1984).

Eschbach et al., "Correction by Erythropoietin (EPO) Therapy of the Anemia of Chronic Renal Failure (CRP) in Sheep," *Clin. Res.* 29(2), 517A (1981).

Eschbach et al., "The Anemia of Chronic Renal Failure in Sheep," *J. Clin. Invest.*, 74(2), 434–441 (Aug. 1984).

Espada et al., "Purification of Human Urinary Erythropoietin," *Fed. Proc.* 41, 1159 (1982).

Fan et al., "Construction and Characterization of Moloney Murine Leukemia Virus Mutants Unable to Synthesize Glycosylated Gag Polyprotein," *Proc. Nat'l. Acad. Sci.* (*USA*), 80, 5965–5969 (1983).

Farber et al., "Translation of mRNA from human kidneys into biologically active erythropoietin following microinjection into *Xenopus laevis*, " *J. Lab. Clin. Med.*, 102, 681 abstract (Nov. 1983).

Faber, "Translation of RNA from Human Kidneys into Biologically Active Erythropoietin Following Microinjection into *Xenopus Laevis* Oocytes," *Clin. Res.*, 31(4), 769A (Nov. 1983).

Faber et al., "Translation of mRNA from Anemic Baboon Kidney into Biologically Active Erythropoietin," *Exp. Hematol.*, 11, Supp. 14, Abstract 101 (1983).

Farber et al., "Translation of mRNA from Human Kidneys into Biologically Active Erythropoietin Following Microinjection into *Xenopus Laevis* Oocytes," *Blood*, 62(5), Supp. No. 1, Abstract 392, 122a (1983).

Fiddes et al., "The Gene Encoding the Common Alpha Subunit of the Four Human Glycoprotein Hormones," *J. Mol. & App. Genetics*, 1, 3–18 (1981).

Fiers et al., "The Human Firbroblast and Human Immune Interferon Genes and Their Expression in Homologous and Heterologous Cells," *Phil. Trans. R. Soc. Lond.*, B299, 29–38 (1982).

Finch, "Erythropoiesis, Erythropoietin, and Iron," *Blood*, 60(6), 1241–1246 (Dec. 1982).

Fischinger et al., "Detection of a Recombinant Murine Leukemia Virus–Related Glycroprotein on Virus–Negative thymona Cells," *Proc. Nat'l . Acad. Sci.* (*USA*), 79(3), 1920–1924 (1981).

Fisher et al., "Cooperative Erythroppietic Assay of Several Steroid Metabolites in Polycythemic Mice," *Steroids*, 30(6), 833–845 (Dec. 1977).

Fisher, "Erythropoietin: Pharmacology, Biogenesis and Control of Production," *Pharmacological Review*, 24(3), 459–508 (1972).

Fisher, "Control of Erythropoietin Production," *Proc. Soc. Exp. Biol. & Med.* 173, 289–305 (1983).

Fisher et al., "Effects of testosterone, colbalt & hypoxia on erythropoietin production in the isolated perfused dog kidney," *Ann. N.Y. Acad. Sci.*, 75–87 (1967).

Garcia et al., "Radioimmunoassay of erythropoietin: circulating levels in normal and polycythemic human beings," *J. Lab. Clin. Med.*, 99, 624–635 (May 1982).

Garcia et al., "Radioimmunoassay of Erythropoietin," *Blood Cells* 5, 405–419 (1979).

Garcia et al., "Immunological Neutralization of Various Erythropoietins," *Proc. Soc. Exptl., Biol. Med.*, 112, 712–714 (1963).

Garoff et al., "Expression of Semliki Forest Virus Proteins from Cloned Complementary DNA. II. The Membrane–spanning Glycoprotein E2 is Ttransported to the Cell Surface Without Its Normal Cytoplasmic Domain," *J. Cell. Biol.*, 97, 652–658 (1983).

Gasser et al., "Expression of abbreviated mouse dihydrofolate reductase genes in cultured hamster cells," *P.N.A.S. (USA)*, 79, 6522–6526 (Nov. 1982).

Gene Screen, New England Nuclear, Catalog No. NEF–972.

Gething et al., "Comparison of Different Eurkaryotic Vectors for the Expression of Hemagglutinin Glycroprotein of Influenza Virus," *Modern Approaches To Vaccines*, pp. 263–268, Chanock et al., eds. Cold Spring Harbor Lab (1984).

Gething et al., "Construction of influenza haemagglutin genes that code for intracellular and secrete forms of the protein," *Nature*, 300, 598–603 (Dec. 16, 1982).

Gething et al., "Cell–surface expression of influenza haemagglutinin form a cloned DNA copy of the RNA gene", *Nature*, 293:62–625 (Oct. 22, 1981).

Gibson et al., "An Evaluation of Serum Erythropoietin Estimation By a Hemagglutination Inhibition assay in the Differential Diagnosis of Polycythemia," *Pathology*, 16, 155–156 (Apr. 1984).

Gluzman, "SV40–Transformed Simian Cells Support the Replication of Early SV40 Mutants," *Cell 23*, 175–182 (Jan. 1981).

Goeddel et al., "Synthesis of human fibroblast interferon by *E. coli," Nucleic Acids Res.*, 8(18), 4057–4074 (1980).

Goeddel et al., "Human leukocyte Interferon Produced by *E. coli*. is biologically active," *Nature*, 287:411–416 (Oct. 2, 1980).

Goldwasser et al., "Erythropoietin: Assay and Study of Its Mode of Action," *Meth. in Enzymol.*, 37, 109–121 (1975).

Goldwasser, "From Protein to Gene to Protein: The Moleclar Biology of Erythropoietin," *Am. J. of Kidney Diseases*, 18(4) *Supp.* 1, 10–13 (Oct. 1991).

Goldwasser, "Biochemical Control of Erythroid Development" *Current Topics in Developmental Biology*, ed. A. Monroy and A.A. Noscona, 173–211, Academic Press, NY (1966).

Goldwasser et al., "On the mechanism of Erythropoietin––induced Differentiation," *J. of Biol. Chem.*, 249(13), 4202–4206 (Jul. 10, 1974).

Goldwasser et al., "The Molecular Weight of Sheep Plasma Erythropoietin," *J. of Biol. Chem.*, 247(16), 5159–60 (Aug. 25, 1972).

Goldwasser et al., "Progress in the purification of erythropoietin," *Ann. N.Y. Acad. Sci.*, 149:49–53 (1963).

Goldwasser et al., "Purification of Erythropoietin," *P.N.A.S. (USA)*, 68(4, 697–698 (Apr. 1971).

Goldwasser et al., "Further purification of sheep plasma erythropoietin", *Bioch. Biophys. Acta*, 64, 487–497 (1962).

Goldwasser, "Some Thoughts on the Nature of Erythropoietin–Responsive Cells," *J. Cell. Physiol.*, 110 (*Supp. 1*), 133–135 (1982).

Goldwasser et al., "An Assay for Erythropoietin in Vitro at the Milliunit Level," *Endocrinology*, 98(2), 315–323 (Aug. 1975).

Goldwasser et al., "Erythropoietin and the differentiation of red blood cells," *Fed. Proc.* 34, 2285–2292 (Dec. 1975).

Goochee et al., "Environmental Effects on Protein Glycosylation," *Biotechnology*, 8, 421–427 (May 1990).

Goochee et al., "The Oligosaccharides of Glycoproteins: Bioprocess Factors Affecting Oligosaccharide Structure and their Effect on Glycoprotein Properties," *Biotechnology*, 9, 1347–1555 (Dec. 1991).

Goodman et al., "Cloning of Hormone Genes from a Mixture of cDNA Molecules," *Meth. in Enzymol.* 68, 75–90 (1979).

Gordon et al., "A plasma extract with erythropoietic activity," *Proc. Soc. Expt. Biol. Med.*, 86:255–258 (1954).

Goto et al., "Production of Recombinant Human Erythropoietin in Mammalian Cells: Host–Cell Dependency of the Biological Activity of the Cloned Glycoprotein," *Bio/Tech.* 6, 67–71 (Jan. 1988).

Gough et al., "Immunoprecipitation of Specific Polysomes Using *Staphylococcus aureus*: Purification of the Immunoglobulin–Chain Messenger RNA from the Mouse Myeloma MPC11," *Biochemistry* 17(25), 5560–5566 (1978).

Gough et al., "Molecular Cloning of cDNA Encoding a Murine Haematopoietic Growth Regulator, Granulocyte–Macrophage Colony Stimulating Factor", *Nature*, 309, 763–767 (1984).

Gouy et al., "Codon Usage in Bacteria: Correlation with Gene Expressivity," *Nucleic Acids Res.* 10, 7055–7074 (1982).

Graham et al., "A New Technique for the Assay of Infectivity of Human Adenovirus 5 DNA," *Virology* 52, 456–467 (1973).

Grantham et al., "Codon catalog usage is a genome strategy modulated for gene expressivity," *Nucleic Acids Res.* 9, r43–74 (1981).

Gray et al., "*Pseudomonas aeruginosa* Secretes and Correctly Processes Human Growth Hormone," *Biotechnology*, 2, 161–165 (Feb. 1984).

Gray et al., "Expression of human immune interferon cDNA in *E. coli* and monkey cells," *Nature*, 295, 503–508 (Feb. 11, 1982).

Green et al., "Immunogenic Structure of the Influenza Virus Hemagglutinin," *Cell,*, 28, 477–487 (Mar. 1982).

Greenwood et al., "The Preparation of ^{131}I–Labelled Human Growth Hormone of High Specific Radioactivity," *Biochem. J.*, 89,, 114–123 (1963).

Grimaldi et al., "Interspersed repeated sequences in the African green monkey genome that are homologous to the human Alu family," *Nucleic Acid Research*, 9(21), 5553–5568 (2981).

Groffen et al. "Isolation of Human Onocogene Sequences (v–fes Homolog) from a Cosmid Library," *Science*, 216, 1236–1138 (Jun. 4, 1982).

Grundmann et al., "Characterization of cDNA coding for human factor XIIIa," *P.N.A.S. (USA)*, 83, 8024–8028 (Nov. 1986).

Grunstein et al., "Colony Hybridization," *Meth. in Enzym.* 68, 379–389 (1979).

Grunstein et al., "Colony hybridization: A method for the isolation of cloned DNAs that contain a specific gene," *P.N.A.S. (USA)*, 72(10), 3961–3965 (Oct. 1975).

Gruss et al., "Expression of simian virus 40–rat preproinsulin recombinants in monkey kidney cells: Use of preproinsulin RNA processing signals," *P.N.A.S. (USA)*, 78(1), 133–137 (Jan. 1981).

Gubler et al., "A simple and very efficient method for generating cDNA libraries," *Gene* 25, 263–269 (1983).
Haddy, "Erythropoietin in sickle cell disease," *Am. Jour. Ped. Hematol./Oncol.*, 4(2), 191–196 (Summer 1982).
Haga et al., "Plasma Erythropoietin Concentrations During the Early Anemia of Prematurity," *Acta. Pediatr. Scand.*, 72, 827–831 (1983).
Hagiwara et al., "Erythropoietin Production in a Primary Culture of Human Renal Carcinoma Cells Maintained in Nude Mice," *Blood*, 63(4), 828–835 (Apr. 1984).
Hamer et al., "Expression of the chromosomal mouse β^{maj}–globin gene cloned in SV40," *Nature*, 281, 35–40 (Sep. 6, 1979).
Hamer et al., "A Mouse Globin Gene Promoter is Functional in SV40," *Cell*, 21, 697–708 (Oct. 1980).
Hammond et al., "Production, Utilization and Excretion of Erythropoietin: I. Chronic Anemias, II. Aplastic Crisis. III. Erythropoietic Effects of Normal Plasma," *Ann. N.Y. Acad. Sci.*, 149, 516–527 (1968).
Hanahan et al., "Plasmid screening at high colony density," *Gene*, 10, 63–67 (1980).
Hartman et al., "Human Influenza Virus Hemagglutinin is Expressed in Monkey Cells Using Simian Virus 40 Vectors," *Proc. Nat'l . Acad. Sci.* (*USA*), 79,233–237 (1982).
Hauser et al., "Inducibility of human β–interferon in mouse L–cell clones," *Nature*, 297, 650–654 (Jun. 24, 1982).
Haynes et al., "Constitutive, long–term production of human interferons by hamster cells containing multiple copies of a cloned interferon gene," *Nucleic Acids Research*, 11(3), 587–607 (1983).
Haynes et al., "Production of a Glycosylated Human Protein by Recombinant DNA Technology," Humoral Factors Host Ref. [Proc. Takeda Sci. Found. Symp. Biosci. (1983)], 1st Meeting Date 1982, 111–29.
Hellmann et al., "Familial erythrocytosis with over–production of erythropoietin," *Clin. Lab. Haemat.*, 5, 335–342 (1983).
Hewick et al., "A Gas–Liquid Solid Phase Peptide and Protein Sequenator," *J. Biol. Chem.*, 256, 7990–7997 (Aug. 1981).
Higashi et al., "Structure and Expression of a Cloned cDNA for Mouse Interferon–β*," *J. Biol. Chem.*, 258(15):9522–9529 (1983).
Hagiashi et al., "Characterization of N–Glycolyineuraminic Acid–containing Gangliosides as Tumor–associated Hanganutziu–Deicher Antigen in Human Colon Cancer," *Cancer Research*, 45, 3796–3802 (1985).
Hirs et al., "Peptides Obtained by Tryptic Hydrolysis of Performic Acid–Oxidized Ribonuclease," *J. Biol. Chem.* 219, 623–642 (1955).
Hokke et al., "Sialylated carbohydrate chains of recombinant human glycoproteins expressed in Chinese hamster ovary cells contain traces of N–glycolyneuraminic acid," *FEBS Letters*, 275, 9–14 (1990).
Hopp et al., "Prediction of protein antigenic determinants from amino acid sequences," *P.N.A.S.* (*USA*), 78(6), 3824–2828 D–7182 (Jun. 1981).
Houghton et al., "The amino–terminal sequence of human fibroblast interferon as deduced from reverse transcripts obtained using synthetic oligonucleotide primers," *Nucleic Acids Res.* 8(9), 1913–1931 (1980).
Huang et al., "Identification of Human Erythropoietin Receptor," *Am. Soci. of Biological Chemists, Am. Assoc. of Immunologists, Fed. Pract.* (*USA*)43(7) Abst. 2770 p. 1891 (1984).
Huang et al., "Characterization of Human Erythropoietin cDNA clones," *Am, Soc. of Biological Chemists, Am. Assoc. of Immunologist, Fed. Pract.* (*USA*) 43(6) Abst. 1795 p. 1724.
Imai et al., "Physicochemical and Biological Comparison of Recombinant Human Erythropoietin with Human Urinary Erythropoietin" *J. Biochem*, 107, 352–359 (1990)
Itakura, et al., "Synthesis and Use of Synthetic Oligonucleotides," *Ann. Rev. Biochem.*, 53, 323–356 (1984).
Ito et al., "Solid phase synthesis of polynucloetides. VI. Further studies on polystyrene copolymers for the solid support," *Nucleic Acids Res.* 10(5), 1755–1769 (1982).
Jacobs et al., "Isolation and characterization of genomic and cDNA clones of human erythropoietin," *Nature*, 313, 806–809 (Feb. 28, 1985).
Jacobsen et al., "Relative effectiveness of phenylhydrazine treatment and hemorrhage in the production of an erythropoietic factor," *Blood*, 11:937–945 (1956).
Jacobson et al., "Role of the kidney in erythropoiesis," *Nature*, 179:633–634 (Mar. 23, 1957).
Jaye et al., "Isolation of human anti–haemophilic factor IX cDNA clone using a unique 52–base synthetic oligonucleotide probe deduced form the amino acid sequnce of bovine factor XI," *Nucleic Acids Res.* 11(8), 2325–2335 (1983).
Jeffreys et al., "Sequence variation and evolution of nuclear DNA in man and the primates," *Phil. Trans. R. Soc. Lond.*, B 292, 133–142 (1981).
Jelkman et al., "Extraction of Erythropoietin from Isolated Renal Glomeruli of Hypoxic Rats," *Exp. Hemotol.*, 11(7), 581–588 (Aug. 1983).
Kaiser et al., "Amphiphilic Secondary Struture: Design of Peptide Hormones," *Science*, 223, 249–255 (1984).
Kajimura et al., "Cloning the Heavy Chain of Human HLA–DR Antigen Using Synthetic Oligodeoxyribonucleotides as Hybridization Probes," *DNA*, 2(3), 175–182 (1983).
Kakidani et al., "Cloning and sequence analysis of cDNA for porcine β–neo–endorphin/dynorphin precursor," *Nature*, 298, 245–249 (Jul. 15, 1982).
Kalmanti, "Correlation of clinical and in vitro erythropoietic responses to androgens in renal failure," *Kidney Int'l.*, 22, 383–391 (1982).
Karn et al., "Novel bacteriophage λ cloning vector," *P.N.A.S.* (*USA*), 77, 5172–5176 (Sep. 1980).
Katsuoka et al., "Erythropoietin Production in Human renal Carcinoma Cells Passaged in Nude Mice and in Tissue Culture," *Gann*, 74, 534–541 (Aug. 1983).
Kaufman et al., "Amplification and Expression of Sequences Cotransfected with a Modular Dihydrofolate Reductase Complementary DNA Gene," *J. Mol. Biol.* 159, 601–621 (1982).
Kaufman et al., "Expression and Amplification of DNA Introduced into Mammalian Cells," *Gene Amplification*, RT Schimke ed., Cold Spring Harbor, New York, 245–250 (1982).
Kennell, "Principles and Practices of Nucleic Acid Hybridization," *Prog. Nucl. Acid Res. Mol. Biol.* 11, 259–301 (1971).
Kenter et al., "Mouse Myeloma Cells That Make Short Immumoglobulin Heavy Chains: Pleitropic Effects on Glycosylation and Chain Assembly," *J. Cell. Biol.*, 98, 2215–2221 (1984).
Kieny et al., "Expression of rabies virus glycoprotein from a recombinant vaccinia virus," *Nature*, 312, 163–166 (1984).

Kimura et al., "A frameshift addition causes silencing o the δ–globin gene in old world monkeys, an anubis," *Nucleic Acids Res*., 11(9):2541–2550 (1983).

Knopf et al., "Cloning and Expression of Multiple Protein Kinase C cDNAs," *Cell* 46, 491–502 (Aug. 15, 1986).

Kohne, "Evolution of Higher–organism DNA," *Quarterly Reviews of Biophysics*, 3:327–375 (1970).

Kondor–Koch et al., "Expression of Semliki Forest Virus Proteins from Cloned Complementary DNA. I. The Fusion Activity of the Spike Glycoprotein," *J. Cell. Biol*., 97, 644–651 (1983).

Konrad, "Applications of Genetic Engineering to the Pharmaceutical Industry," *Ann. N.Y. Acad. Sci*., 413, 12–22 (1983).

Konwalinka et al., "A Miniaturized Agar Culture System for Cloning Human Erythropoietic Progenitor Cell," *Exp. Hematol*., 12, 75–79 (1984).

Korman, "cDNA clones for the heavy chain of HLA–DR antigens obtained after immunopurification of polysomes by monoclonal antibody," *P.N.A.S*. (*USA*), 79, 1844–1848 (Mar. 1982).

Kornblihtt et al., "Isolation and characterization of cDNA clones for human and bovine fibronectins," *P.N.A.S*. (*USA*), 80, 3218–3222 (Jun. 1983).

Kramer et al., "Comparisons of the Complete Sequences of Two Collagen Genes from Caenorhabditis elegans," *Cell* 30, 599–606 (Sep. 1982).

Krane, "The Role of Erythropoietin in the Anemia of Chronic Renal Failure," *Henry Ford Hosp. Med. J*., 31(3), 177–181 (1983).

Krystal, "A Simple Microassay for Erythropoietin Based on ^{3}H–Thymidine Incorporation into Spleen cessl from Phenylhydrazine Treated Mice," *Exp. Hematol*., 11(7), 649–660 (Aug. 1983).

Kuhn et al., "Gene Transfer, Expression, and Molecular Cloning of the Human Transferrin Receptor Gene," *Cell*, 37, 95–103 (1984).

Kurachi et al., "Isolation and characterization of a cDNA coding for human factor IX," *P.N.A.S*. (*USA*), 79, 6462–6464 (Nov. 1982).

Kuratowska et al., "Studies on the production of erythropoietin by isolated perfused organs," *Blood*, 18:527–534 (1961).

Kurtz, "A New candidate for the regulation of erythropoiesis: Insulin–like growth factor I," *FEBS Letters*, 149(1), 105–105 (Nov. 1982).

Kyte et al., "A Simple Method for Displaying the Hydropathic Character of a Protein," *J. Mol. Biol*., 157, 105–132 (1982).

Lai et al., "Ovalbumin is synthesized in mouse cells transformed with the natural chicken ovalbumin gene," *P.N.A.S*. (*USA*), 77(1), 244–248 (Jan. 1980).

Lai et al., "Structural Characterization of Human Erythropoietin," *J. of Biol. Chem*., 261, 3116–3121 (Mar. 5, 1986).

Lai, "Technical improvements in Protein Microsequencing," *Analtytica Chimica Acta*, 163, 243–248 (1984).

Lange et al., "Application of erythropoietin antisera to studies of erythropoiesis," *Ann. N.Y. Acad. Sci*., 149:281–291 (1968).

Lappin et al., "The Effect of Erythropoietin and Other Factors on DNA synthesis by Mouse Spleen Cells," *Exp. Hematol*., 11(17), 661–666 (Aug. 1983).

Lasky et al., "Production of an HSV Subunit Vaccine by Genetically Engineered Mammalian Cell Lines," *Modern Approaches to Vaccines*, pp. 189–194, Chanock et al., eds. Cold Spring Harbor Lab. (1984).

Lathe, "Synthetic Oligonucleotide Probes Deduced from Amino Acid Sequence Data," *J. Mol. Biol*. 183, 1–12 (1985).

Laub and Ritter, "Expression of the Human Insulin Gene and cDNA in a Heterologous Mammalian System," *J. Biol. Chem*., 258(10), 6043–6050 (May 25, 1983).

Laub et al., "Synthesis of Hepatitis B Surface Antigen in Mammalian Cells: Expression of the Entire Gene and the Coding Region," *J. Viol*., 48(1):271–280 (1983).

Lauffer et al., "Topology of signal recognition particle receptor in endoplasmic reticulum membrane," *Nature*, 318, 334–338 (Nov. 28, 1985).

Lawn et al., "The Isolation and Characterization of Linked δ–and β–Globin Genes from a Cloned Library of Human DNA," *Cell*, 15, 1157–1174 (Dec. 1978).

Ledeen et al., "Gangliosides: Struture, Isolation, and Analysis," *Methods in Enzymology*, 83 (Part D), 139–191 (1982).

Lee–Huang, "The Erythropoietin Gene," *Oncogenes, Genes and Growth Factors*, Chap. 7, pp. 199–222, ed. Gordon Garaff, John Wiley & Sons, Inc. (1987).

Lee–Huang, "Cloning of Human Erythropoietin," *Biophysical J*., 45(Part 2 of 2), ABT. M–PM–A12, p. 30a (1984).

Lee–Huang, "Monoclonal Antibodies to Human Erythropoietin," *Abstract No. 1463, Fed. Proc*., 41, 520 (1982).

Lee–Huang, "A New Preparative Method for Isolation of Human Erythropoietin With Hydrophobic Interaction Chromatography," *Blood*, 56(4), 620–624 (Oct. 1980).

Lee–Huang, "Cloning and Expression of Human EPO cDNA in *E. coli*," *P.N.A.S*. (*USA*), 81, 2708–2712 (May 1984).

Lerner et al., "Chemically synthesized peptides predicted from the nucleotide sequence of the hepatitis B virus genome elicit antibodies reactive with the native envelope protein of Dane particles," *P.N.A.S*.(*USA*), 78(6), 3403–3407 (Jun. 1981).

Lerner, "Synthetic Vaccines," *Scientific American*, 248(2), 66–74 (1983).

Lerner et al., "Antibodies to Chemically Synthesized Peptides Predicted from DNA Sequences as Probes of Gene Expression," *Cell*, 23, 309–310 (Feb. 1981).

Lewin *Genes*, 1983, John Wiley & Sons, p. 307.

Lin et al., "Cloning and expression of the human erythropoietin gene," *P.N.A.S*. (*USA*), 82, 7580–7584 (Nov. 1985).

Lin et al., "Monkey erythropoietin gene: cloning, expression and comparison with the human erythropoietin gene," *Gene*, 44, 201–209 (1986).

Lin et al., "Cloning of the Monkey EPO Gene," Abstract, *J. Cell. Bioch., Suppl*. 8B, p. 45 (Mar. 31–Apr. 24, 1984).

Lin et al., "Cloning and Expression of Monkey and Human Erythropoietin," *Exp. Hematol*. 12, 357 (1984).

Lipschitz et al., "Effect of Age on Hematopoiesis in Man," *Blood*, 63(3), 502–509 (Mar. 1983).

LKB Technical Bulletin #2217.

Maniatis et al., "The Isolation of Structural Genes from Libraries of Eucaryotic DNA," *Cell* 15, 687–701 (Oct. 1978).

Maniatis et al., "Molecular Cloning, a Laboratory Manual", pp. 5, 197–199, 392–393, 479–487, 493–503 Cold Springs Harbor, N.Y. (1982).

Markoff et al., "Glycosylation and Surface Expression of the Influenza Virus Neuraminidase Requires the N–Terminal Hydrophobic Region," *Molecular and Cellular Biology*, 4(1), 8–16 (1984).

Marshall, "Glycoproteins," *Annual Review of Biochemistry*, Snell et al., eds., vol. 41, pp. 673–702, Annual Reviews Inc., Palo Alto, California (1972).

Martial et al., "Human Growth Hormones: Complementary DNA Cloning and Expression in Bacteria," *Science*, 205, 601–606 (Aug. 10, 1979).

Mason et al., "Complementary DNA sequences of ovarian follicular fluid inhibin show precursor structure and homology with transforming growth factor–β," *Nature* 318, 659–663 (Dec. 1985).

Maurer, "Immunochemical Isolation of Prolactin Messenger RNA," *J. Biol. Chem* 255(1), 854–859 (Feb 10, 1980).

Maxam et al., "Sequencing End–Labeled DNA with Base–Specific Chemical Cleavages," *Methods in Enzymol.* 65, 499–560 (1980).

McCormick et al., "Regulated Expression of Human Interferon Genes In Chinese Hamster Ovary Cells," *DNA*, 2(1): 86 Abst 86 (1983).

McCormick et al., "Inducible Expression of Amplified Human Beta Interferon Genes in CHO Cells," *Mol. Cell. Biol.*, 4(1):166–172 (Jan. 1984).

McGonigle et al., "Erythropoietin deficiency and inhibition of erythropoiesis in renal insufficiency," *Kidney Int'l.*, 25(2), 437–444 (1984).

Meier et al., "Alpha$_1$and Beta$_2$–Adrenergic Receptors Co–Expressed on Cloned MDCK Cells are Distinct Glycoproteins," *Biochem& Biophys. Res. Comm.*, 118(1), 73–81 (1984).

Mellon et al., "Identification of DNA Sequences Required for transcription of the human α1–Globin Gene in a New SV40 Host–Vector System," *Cell*, 27, 279–288 (Dec. 1981).

Mellor et al., "Expression of Murine H–2K^b histocompatibility antigen in cells transferred with cloned H–2 genes," *Nature*, 298:529–534 (Aug. 1982).

Messing, "New M13 Vectors for Cloning," *Methods in Enzymology*, 101, 20–78 (1983).

Metcalf et al., "Effect of Purified Bacterially Synthesized Murine Multi–CSF (IL–3) on Hematopoiesis in Normal Adult Mice," *Blood*, 68(1), 46–57 (Jul. 1986).

Metcalf et al., "Quantitative Responsiveness of Murine Hemopoietic Populations in vitro and in vivo to Recombinant Multi–CSF (IL–3)," *Exp. Hematol.*, 15, 288–295 (1987).

Methods in Yeast Genetics, Cold Spring Harbor Lab, Cold Spring Harbor, NY, p. 62 (1983).

Miller et al., "Plasma levels of immunoreactive erythropoietin after acute blood loss in man," *Brit. J. Haematol.*, 52, 545–549 (1982).

Mirand, "Extra–renal and renal control of erythropoietin production ," *Ann. N.Y. Acad. Sci.*, 149:94–106 (1968).

Mirand et al., "Current studies on the role of erythropoietin on erythropoiesis", *Ann. N.Y. Acad. Sci.*, 77:677–702 (1959).

Mladenovic et al., "Anemia of Chronic Renal Failure (CRF) in the Sheep: Response to Erythropoietin (EP) In Vivo and In Vitro," *Blood*, 58(5), Suppl. 1,99a (1981).

Montgomery et al., "Identification and Isolation of the Yeast Cytochrome c Gene," *Cell*, 14, 676–680 (Jul. 1978).

Moriarty et al., "Expression of the Hepatitis B Virus Surface Antigen Gene in Cell Culture by using a Simian Virus 40 Vector," *P.N.A.S.* (USA), 78(4):2606–10 (Apr. 1981).

Moriuchi et al., "Thy–1 cDNa sequence suggest a novel regulatory mechanism," *Nature*,301, 80–82 (Jan. 1983).

Morrison, "Bioprocessing in Space—an Overview", *The World Biotech Report*, vol. 2:USA, 557–571 (1984).

Munjaal et al., "A cloned calmodulin structural gene probe is complementary to DNA sequence from diverse species," *P.N.A.S.* (*USA*), 78(4):2330–2334 (Apr. 1981).

Murphy et al., "The Role of Glycoprotein Hormones in the Regulation of Hematopoiesis," *Acta. Haematologica Japonica*, 46(7), 1380–1396 (Dec. 1983).

Myers et al., "Construction and Analysis of Simian Virus 40 Origins Defective in Tumor Antigen Binding and DNA Replication," *P.N.A.S.* (*USA*), 77, 6491–6495 (Nov. 1980).

Myklebost et al., "The Isolation and Characterization of cDNA clones for Human Apolioprotein CII ," *J. of Biol. Chem.*, 259(7), 4401–4404 (Apr. 10, 1984).

Naets, "The role of the kidney in erythropoiesis,"*J. Clin. Invest.*, 39:102–110 (1960).

Nagata et al., "Synthesis in *E. Coli* of a polypeptide with human leukocyte interferon activity," *Nature*, 284, 316–320 (Mar. 27, 1980).

Nakao et al., "Erythropoiesis in anephric or kidney transplated patients," *Israel J. Med. Sci.*, 7:986–989 (Jul.–Aug. 1971).

Nathan et al., "Erythropoietin and the Regulation of Erythropoiesis," *New Eng. J. Med.*, 308(9), 520–522 (Mar. 3, 1983).

Naughton et al., "Evidence for an Erythropoietin–Stimulating Factor in Patients with Renal and Hepatic Disease," *Act. Haemat.*, 69, 171–179 (1983).

Naughton et al., "Evidence for a Hepatic–Renal Antagoinism in the Production of Hepatic Erythropoietin," *Ann. Clin. Lab. Sci.*, 13(5), 432–438 (1983).

Nayak et al., "Characterization of Influenza Virus Glycoproteins Expressed from Cloned cDNAs in Prokaryotic and Eukaryotic Cells," *Modern Approaches To Vaccines*, pp. 165–172, Chanock et al., eds., Cold Spring Harbor Lab. (1984).

Neeser et al., "A Quantitative Determination by Capillary Gas–Liquid Chromatography of Neutral and Amino Sugars (as O–Methyloxime Acetates), and a Study on Hydrolytic Conditions for Glycoproteins and Polysaccharides In Order to Increase Sugar Recoveries," *Anal. Biochem.*, 142, 58–67 (1984).

Newman et al., "Selection and Properties of a Mouse L–Cell Transformant Expressing Human Transferrin Receptor," *Nature*, 304, 643–645 (1983).

Nigg et al., "Immunofluorescent localization of the transforming protein of *Rous sarcoma* virus with antibodies against a synthetic src peptide," *P.N.A.S.* (*USA*), 79, 5322–5326 (Sep. 1982).

Nimtz et al., "Structures of sialylated oligosaccharides of human erythropoietin expressed in recombinant BHK–21 cells," *Eur. J. Biochem*, 213, 39–56 (1993).

Noda et al., "Primary structure of α–subunit precursor of Torpedo californica acetylcholine receptor deduced from cDNA sequence," *Nature*, 299, 793–797 (Oct. 28, 1982).

Noda et al., "Cloning and sequence analysis of cDNA for bovine adrenal preproenkephalin," *Nature*, 295, 202–206 (Jan. 21, 1982).

Noyes et al., "Detection and partial sequence analysis of gastrin mRNA by using an oligodeoxynucleotide probe," *P.N.A.S.* (*USA*), 76(4), 1770–1774 (Apr. 1979).

Nussinov, "Eukaryotic Dinucleotide Preference Rules and their Implications for Degenerate Codon Usage," *J. Mol. Biol.*, 149, 125–131 (1981).

Olge et al., "Production of erythropoietin in vitro: a review," In Vitro, 14(11), 945–949 (1978).

Ohkubo et al., "Cloning and sequence analysis of cDNA for rat angiotensinogen," *P.N.A.S.* (*USA*), 80, 2196–2200 (Apr. 1983).

Ohno et al., "Inducer–responsive expression of the cloned human interferons β1 gene introduced into cultured mouse cells," *Nucleic Acids Res.*, 10(3), 967–976 (1982).

Okayama et al., "High–Efficiency Cloning of Full–Length cDNA," *Mol. & Cell. Biol.*, 2(2), 161–170 (Feb. 1982).

Ovchinnikov et al., "The Primary Structure of *Escherichia coli* RNA Polymerase,"*J. Biochem.*, 116, 621–629 (1981).

Paabo et al., "Association Between Transplantation Antigens and a Viral Membrane Protein Synthesized from a Mammalian Expression Vector," *Cell*, 35, 445–453 (1983).

Palmiter et al., "Metallothionein–Human GH Fusion Genes Stimulate Growth of Mice," *Science*, 222, 809–814 (Nov. 18, 1983).

Pankratz et al., "A Simple 3–Step Procedure for Purifying Baboon Urinary Erythropoietin to Apparent Homogeneity," *Exp. Hematol.*, 11, Supp. 14, Abst. 102 (1983).

Papayannopoulou et al., "On the In Vivo Action of Erythropoietin: A Quantitative Analysis," *J. of Clin. Investigation*, 51, 1179–1185 (1972)

Parekh et al., "N–Glycosylation and in vitro Enzymatic Activity of Human Recombinant Tissue Plasminogen Activator Expressed in Chinese Hamster Ovary Cells and a murine Cell Line," *Biochemistry*, 28, 7670–7679 (1989).

Pellicer et al., "Altering Genotype and Phenotype by DNA–Mediated Gene Transfer," *Science*, 209, 1414–1422 (Sep. 19, 1980).

Pennathur–Das et al., "Evidence for the Presence of CFU–E with Increased In Vitro Sensitivity to Erythropoietin in Sickle Cell Anemia," *Blood*, 63(5), 1168–71 (May 1984).

Pennica et al., "Cloning and expression of human tissue–type plasminogen activator cDNA in *E–coli,"Nature*, 301, 214–221 (Jan. 20, 1983).

Pennica et al., "Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin," *Nature*, 312, 724–728 (Dec. 27, 1984).

Pitha et al., "Induction of human β–interferon synthesis with poly (rI•rC) in mouse cells transfected with cloned cDNA plasmids," *P.N.A.S.* (*USA*), 79, 4337–7341 (Jul. 1982).

"Points to Consider in the Characterization of Cell Lines Used to Produced Biologics," Jun. 1, 1984, Office of Biologics Research Review, Center for Drugs & Biologics, U.S. Food & Drug Administration (Section A. Part 2).

Powell et al., "Human erythropoietin gene: High level expression in stably transfected mammalian cells and chromosome localization," *P.N.A.S.* (*USA*), 83, 6465–6469 (Sep. 1986).

Prooijen–Knegt, "In Situ Hybridization of DNA Sequences in Human Metaphase Chromosomes Visualized by an Indirect Fluorescent Immunocytochemical Procedure," *Exp. Cell Res.*, 141, 397–407 (1982).

Ramabhadran et al., "Synthesis and Glycosylation of the Common α Subunit of Human Glycoprotein Hormones in Mouse Cells," *Proc. Nat'l. Acad. Sci.* (*USA*), 81, 6701–6705 (1984).

Rambach et al., "Acid Hydrolysis of Erythropoietin," *Proc. Soc. Exp. Biol.*, 99, 482–483 (1958).

Ravetech et al., "Evolutionary approach to the question of immunoglobulin heavy chain swithching: Evidence from cloned human and mouse genes," *P.N.A.S.* (*USA*), 77(11), 6734–6738 (Nov. 1980).

Recny et al., "Structural Characterization of Natural Human Urinary and Recombinant DNA–derived Erythropoietin," *J. Biol. Chem.*, 262(35), 17156–17163 (Dec. 15, 1987).

Reilly et al., "Use of synthetic oligonucleotides to clone genomic DNA: isolation of a $tRNA^{Phe}$ gene from mouse," *DNA*, 1:192 (1982).

Resegotti et al., "Treatment of aplastic aneamia with methoenolone, stanozolol and nandrolone," *Panminerva Medica*, 23, 243–248 (1981).

Reyes et al., "Identification of an H–$2K^b$–Related Molecule by Molecular Cloning", *Immuogenetics*, 14, 383–392 (1981).

Reyes et al., "Isolation of a cDNA clone for the murine transplantation antigen H–$2K^b$," *P.N.A.S.* (*USA*), 79, 3270–3274 (May 1982).

Rigby, "Expression of cloned genes in eukaryotic cell using vector systems derived from viral replicons," *Genetic Engineering*, R. Williamson, ed., 3:83–140, Academic Press, London 1982.

Riggs et al., "Synthetic DNA and Medicine," *Am. J. Hum. Genet.*, 31, 531–538 (1979).

Ringold et al., "Co–Expression and Amplification of Dihydrofolate Reductase cDNA and the *Escherichia coli* XGPRT Gene in Chinese Hamster Ovary Cells," *J. Mol. & Appl. Genetics*, 1(3), 165–175 (1981).

Robson et al., "Polysome immunoprecipitation of phenylalanine hydroxylase mRNA from rat liver and cloning of its cDNA," *P.N.A.S*, (*USA*), 79, 4701–4705 (Aug. 1982).

Roh et al., "Plasma Disappearance of I^{125}labeled Human Uninary Erythorpoietin in Rabbits," *Fed. Proc.*, 29(2), 782 Abst. 3030 (1970).

Rose et al., "Expression from Cloned cDNA of Cell–Surface Secreted Forms of the Glycoprotein of Vesicular Stomatitis Virus in Eucaryotic Cells," *Cell*, 30, 753–762 (1982).

Ross et al., "Phosphotyrosine–containing proteins isolated by affinity chromatography with anitbodies to a synthetic hapten," *Nature*, 294, 654–656 (Dec. 17, 1981).

Roth et al., "Influenza Virus Hemagglutinin Expression Is Polarized in Cells Infected with Recombinant SV40 Viruses Carrying Cloned Hemagglutinin DNA", *Cell*, 33, 435–443 (1983).

Rothmann et al., "Erythropoietin–Dependent Erythrocytosis Associated with Hepatic Angiosarcoma," *J. Surg. Oncol.*, 20, 105–108 (1982).

Saito et al., "Translation of Human Erythropoietin–mRNAs," *Exp. Hematol.*, 11(14), 228 (1983).

Saito et al., "In Vitro Assay of Erythropoietin: Simple Determination in a Small Amount of Human Serum Samples," *Jap. J. Med.*, 23(1), 16–21 (Feb. 1984).

Sambrook et al., "Expression of Proteins on the cell Surface Using Mammalian Vectors," *Experimental Manipulation of Gene Expression*, pp. 225–246, Acad. Press. (1983).

Sanger et al., "DAN Sequencing with chain–terminating inhibitors," *P.N.A.S.* (*USA*), 74, 5463–5467 (Dec. 1977).

Sasaki, "Carbohydrate Structure of Erythropoietin Expressed in Chinese Hamster Ovary Cells by a Human Erythorpoietin cDNA," *J. Biol. Chem.*, 262(25), 12059–12070 (Sep. 5, 1987).

Sasaki, "Isolation of erythropoietin by monoclonal antibody," *Biomed. Biochim. Acta.*, 42(11/12), S202–206 (1983).

Scahill et al., "Expression and characterization of the product of a human immune interferon cDNA gene in Chinese hamster ovary cells," *Proc. Nat'l. Acad. Sci.* (*USA*), 80, 4654–4658 (1983).

Schulze et al., "Identification of the cloned gene for the murine transplantation antigen H–2K^b by hybridization with synthetic oligonucleotides," *Mol. & Cell Biol.*, 3(4), 750–755 (Apr. 1983).

Schwartz et al., "Severe Anemia as a Manifestation of Metastic Jugular Paraganglioma," *Arch Otolaryngol*, 109, 269–272 (Apr. 1983).

Seeburg et al., "Synthesis of growth hormone by bacteria," *Nature*, 276, 795–798 (Dec. 1978).

Seki et al., "Isolation of a genomic clone containing structural information for the DRα subunit", *Fed. Proc.*, 41:365 (1982)/Chemistry and Molecular Biology of Ia/Dr Antigens Abstract 563 (1982).

Shahidi, "Androgens and Erythropoiesis," *New. Eng. J. of Med.*, 289, 72–80 (Jul. 12, 1973).

Sherwood et al., "Erythropoietin Titers in Sickle Cell Disease & Chronic Renal Failure," *Blood Suppl. 1*, 58, Abstract 105 (1981).

Sherwood et al., "Estraction of erythropoietin from normal kidneys," *Endo*, 103(3) 866–870 (1978).

Sherwood et al., "A Radioimmunoassay for Erythropoietin," *Blood*, 54(4), 885–893 (Oct. 1979).

Shiramizu et al., "Human Renal Carcinoma Cells Secreting Erythropoietin in vivo and in vitro," *Blood*, 78(10), Supp. 1 (Nov. 15, 1991).

Shinger–Sam et al., "Isolation of a cDNA clone for human X–Linked 3–phosphoglycerate kinase by use of a mixture of synthetic oligodeoxyribonucleotides as a detection probe," *P.N.A.S.* (*USA*), 80, 802–806 (Feb. 1983).

Smith et al., "Construction and characterization of an infectious vaccinia virus recombinant that expresses the influenza hemagglutinin gene and induces resistance to influenza virus infection in hamsters," *Proc. Nat'l. Acad. Sci.* (*USA*), 80, 7155–7159 (1983).

Southern et al., "Transformation of Mammalian Cells to Antibiotic Resistance with a Bacterial Gene Under Control of the SV40 Early Region Promoter," *J. Mol. Appl. Genet.*, 1(4), 327–341 (1982).

Southern, "Detection of Specific Sequences Among DNA Fragments Separated by Gel Electrophoresis," *J. Mol. Biol.*, 98, 503–517 (1975).

Spellman et al., "Carbohydrate Structure of Recombinant Soluble Human CD4 Expressed in Chinese Hamster Ovary Cells," *Biochemistry*, 30(9), 2395–2406 (1991).

Spellman et al., "Carbohydrate Structure of Human Tissue Plasminogen Activator Expressed in Chinese Hamster Ovary Cells," *J. of Biol. Chem.*, 264(24), 14100–14111 (Aug. 26, 1989).

Srinivas et al., "Membrane Association and Defective Transport of Spleen Focus–forming Virus Glycoproteins," *J. Biol. Chem.*, 258, 14718–14724 (1983).

Stanley, "Surface Carbohydrate Alteration of Mutant Mammalian Cells Selected for Resistance to Plant Lectins," *The Biochemistry of Glycoproteins and Proteoglycans*, Chapter 4:161–189, Lennarz ed., Plenum Press (1980).

Steck et al., "Cell Surface Properties of Spontaneously Metastasizing Rat Mammary Adenocarcinoma Cell Clones," *Transplantation Proceedings*, 16, 355–360 (1984).

Storring et al., "The International Standard for Recombinant DNA derived Erythropoietin: Collaborative Study of four recombinant DNA derived erythropoietins and two highly purified human urinary erythropoietins," *J. of Endo.*, 134, 459–84 (1992).

Strickland, "Occurrence of Sulfate on the N–Linked Oligosaccharides of Human Erythropoietin," *J. of Cellular Biochemistry*, Suppl. 16D, Abstract No. P324 (1992).

Sue et al., "Site–specific antibodies to human erythropoietin directed toward the NH_2–terminal region," *Proc. Nat. Acad. Sci.* (*USA*), 80, 3651–3655 (1983).

Suggs et al., "Use of Synthetic Oligodeoxyribonucleotide for the Isolation of Specific Cloned DNA Sequences," *Developmental Biology Using Purified Genes*, 683–693 (D. Brown, Ed., 1981).

Suggs et al., "Use of synthetic oligonucleotides as hybridization probes: Isolation of cloned cDNA sequences for human B_2–microglobulin," *P.N.A.S.* (*USA*), 78, 6613–6617 (1981).

Sveda et al., "Functional expression in primate cells of cloned DNA coding for the hemagglutinin surface glycoprotein of influenza virus," *Pros. Nat'l. Acad. Sci.* (*USA*), 78(10):5488–5492 (Sep. 1981).

Sytowski et al., "The Biochemistry of Erythropoietin: An Approach to its mode of Action," *Exp. Hematol.*, 8(Supp. 8), 52–63 (1980).

Sytowski et al., "A Novel Radioimmunoassay for Human Erythropoietin Using a Synthetic NH_2–Terminal Polypeptide and Anti–Peptide Antibodies," *J. Immunol. Methods*, 69, 181–186 (1984).

Szostak et al., "Hybridization with Synthetic Oligonucleotides," *Meth. in Enzymol.*, 68, 419–429 (1979).

Takeuchi et al., "Relationship between sugar chain structure and biological activity of recombinant human erythropoietin produced in Chinese hamster ovary cells," *P.N.A.S.* (*USA*), 86, 7819–7822 (Oct. 1989).

Takeuchi, "Comparative Study of the Asparagine–linked Sugar Chains of Human Erythropoietin Purified from Urine and the Culture Medium of Recombinant Chinese Hamster ovary Cells," *J. Biol. Chem.*, 263(8), 3657–3663 (Mar. 15, 1988).

Talmadge et al., "Eukaryotic Signal Sequence Transports Insulin Antigen in *Escherichia coli*," *P.N.A.S. USA 77*(66), 3369–3373 (Jun. 1980).

Tambourin et al., "Production of erythropoietin–like activity by a murine erythroleukemia cell line," *P.N.A.S. USA*), 80, 6269–6273 (1983).

Taniguchi et al., "Structure and expression of a cloned cDNA coding for the hemagglutinin surface glycoprotein of influenza virus," *Nature*, 302:305–310 (Mar. 24, 1983).

Taub et al., "An improved method for preparing large arrays of bacterial colonies containing plasmids for hybridization: in situ purification and stable binding of DNA on paper filters," *Chemical Abstracts*, 97(23), 164, Abstract No. 194002y (Dec. 12, 1982).

Taub et al., "An Improved Method for Preparing Large Arrays of Bacterial Colonies Containing Plasmids for Hybridization: In Situ Purification and Stable Binding of DNA on Paper Filters," *Anal. Biochem.*, 126, 222–230 (1982).

Testa et al., "Role of Purified Erythropoietin in the Amplification of the Erythroid Compartment," *Exp. Hematol.*, 8(Supp. 8), 144–152 (1980).

Tong et al., "The Formation of Erythrocyte Membrance Proteins during Erythropoietin–induced Differentiation," *J. Biol. Chem.*, 256(24), 12666–12672 (Dec. 25, 1981).

Toole et al., "Molecular cloning of a cDNA encoding human antihaemophilic factor," *Nature*, 312, 342–347 (Nov. 8, 1984).

12(12), 5049–5059 (1984).

Tramontano et al., "Statistical evaluation of the coding capacity of complementary DNA strands," *Nucleic Acids Research*, 12(12), 5049–5059 (1984).

Tsuda et al., "Comparative Structural Study of N–Linked Oligosaccharides of Urinary and Recombinant Erythropoietins" *J. Amer. Chem. Soc.*, 27(15), 5646–5654 (1988).

Udupa et al., "Erythropoiesis in the aged mouse," *J. Lab. Clin. Med.*, 103(4), 574–580 & 581–588 (1984).

Ullrich et al., "Rat Insulin Genes: Construction of Plasmids Containing the Coding Sequence," *Science*, 196, 1313–1319 (Jun. 17, 1977).

Ullrich et al., "Isolation of the Human Insulin–like Growth Factor I Gene Using a Single Synthetic DNA Probe," *EMBO J.*, 3(2):361–364 (1984).

Ullrich et al., "Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells," *Nature*, 309, 418–425 (May 31, 1984).

Ullrich et al., "Insulin–like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity," *EMBO J.*, 5(10), 2503–2512 (1986).

Ullrich et al., "Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes," *Nature*, 313, 756–761 (Feb. 28, 1985).

Urabe et al., "The Influence of Steroid Hormone Metabolities on the In Vitro Development of Erythroid Colonies Derived from Human Bone Marrow," *J. Exp. Med.*, 149, 1314–1325 (Jun 1979).

Urlaub et al., "Isolation of Chinese Hamster cell mutants deficient in dihydrofolate reductase activity," *Proc. Nat. Acad. Sci*, (*USA*), vol. 77(7), 4216–4220 (Jul. 1980).

Van der Ploeg et al., "DNA Methylation in the Human vδβ–Globin Locus in Erythroid and Nonerythroid Tissues," *Cell*, 19, 947–958 (Apr. 1980).

Van Stone et al., "Effect of erythropoietin on anemia of peritoneally dialyzed anephric rats," *Kidney Int'l.*, 15, 370–375 (1979).

Varki, "Diversity in the sialic acids," Oxford University Press, 25–40, (1992).

Vedovato et al., "Erythropoietin Levels in Heterozygous Beta–Thalassemia," *Acta. Haematol.*, 71, 211–213 (1984).

Vichinsky et al., "Inadequate erythroid response to hypoxia in cystic fibrosis," *J. Pediatr.*, 105(1), 15–21 (Jul. 1984).

Vieira et al., "The pUC plasmids, an M13mp7–derived system for insertion mutagenesis and sequencing with synthetic universal primers," *Gene*, 19, 259–268 (1982).

Villasante et al., "Binding of microtubule protein to DNA and chromatin: possibility of simultaneous linkage of microtubule to nucleic acid and assembly of the microtubule structure," *Nucleic Acids Res*, 9(4), 895 (1981).

Walker et al., *Techniques in Molecular Biology*, Macmillan Pub. Co., N.Y., p. 280 (1983).

Wallace et al., "Hybridization of synthetic oligodeoxyribonucleotides to Phi–chi 174 DNA: the effect of single base pair mismatch," *Nuc. Acids Res.*, 6(11), 3543–3557 (1979).

Wallace et al., "Directed Deletion of a Yeast Transfer RNA Intervening Sequence," *Science*, 209:1396–1400 (Sep. 19, 1980).

Wallace et al., "Oligonucleotide Directed Mutagenesis of the Human β–globin gene: A General Method for Producing Specific Point Mutations in cloned DNA," *Nucleic Acids Research*, 9(15):3647–3657 (1981).

Wallace et al., "The use of synthetic oligonucleotides as hybridization probes. II. Hybridization of oligonucleotides of mixed sequence to rabbit β–globin DNA," *Nuc. Acids Res.*, 9(4), 879–894 (1981).

Wallace et al., "A set of synthetic oligodeoxyribonucleotide primers for DNA sequencing in the plasmid vector pBR322," *Gene*, 16, 21–26 (1981).

Wallis et al., "The isolation of cDNA clones for human apolipoprotein E and the detection of apoE RNA in hepatic and extra–hepatic tissues," *EMBO J.*, 2, 2369–2373 (1983).

Walter et al., "Antibodies specific for the carboxy– and amino–terminal regions of simian virus 40 large tumor antigen," *P.N.A.S.* (*USA*), 77(9), 5197–5200 (Sep. 1980).

Walter et al., "Antibodies specfic for the polyoma virus middle–size tumor antigen," *P.N.A.S.* (*USA*), 78, 4882–4886 (Aug. 1981).

Wang et al., "Some Chemical Properties of Human Erythropoietin", *Endo*116(6), 2286–2292 (1985).

Wang et al., "Renal and extrarenal erythropoietin production in male and female rats of various ages," *J. Lab. Clin. Med.*, 79(2),181–186 (Feb. 1972).

Weiland et al., "In vivo Activity of Asialo–Erythropoietin in Combination with Asialo–Glycoproteins," *Blut*, 44(3), 173–175 (1982).

Weiss et al., "Characterization of a monoclonal antibody to human erythropoietin," *P.N.A.S.* (*USA*), 79, 5465–5469 (1982).

Weiss et al., "Studies of the pathogenesis of anemia of inflammation: Mechanism of impaired erythropoiesis," *Am. J. Vet. Res.*, 44(10), 1832–1835 (Oct. 1983).

Weissman et al., "Structure and expression of human IFN–α Genes," *Phil. Trans. R. Soc. Lond.*, B299, 7–28 (1982).

White et al., "Studies on Erythropoietin," *Recent Progr. Hormone Res.*, 16:219–262 (1960).

White et al., "Haemagglutinin of influenza virus expressed from a cloned gene promotes membrane fusion," *Nature*, 300, 658–659 (1982).

Whitehead et al., "Use of a cDNA Clone for the Fourth Component of Human Complement (C4) for Analysis of a Genetic Deficiency of C4 in Guinea Pig", *PNAS* (*USA*), 80:5387–5391 (Sep. 1983).

Wiaderkiewicz et al., "Mismatch and blunt to protruding–end joining by DNA ligases," *Nucleic Acids Res.*, 15(19), 7831–7848 (1987).

Wickens et al., "Expression of a chicken chromosomal ovalbumin gene injected into frog oocyte nuclei," *Nature* 285:628–634 (Jun. 26, 1980).

Wide et al., "Molecular charge heterogeneity of human serum erythropoietin," *British J. Haemat.*, 76, 121–127 (1990).

Wiktor et al., "Protection from rabies by a vaccinia virus recombinant containing the rabies virus glycoprotein gene," *Proc. Nat'l . Acad. Sci.* (*USA*), 81, 7194–7198 (1984).

Wong et al., "Synthetic peptide fragment of src gene product inhibits the src protein kinase and crossreacts immunologically with avian onc kinases and cellular phosphoproteins," *P.N.A.S.* (*USA*), 78(12), 7412–7416 (Dec. 1981).

Wong et al., "Interferon–γ Induces Enhanced Expression of Ia And H–2 Antigens on B Lymphoid, Macrophage, and Myeloid Cell Lines," *J. Immun.*, 131(2):788–793 (Aug. 1983).

Woo, "A Sensitive and Rapid Method for Recombinant Phage Screening," *Methods in Enzymology*, 68, 389–395 (1979).

Wood et al., "Expression of active human factor VIII from recombinant DNA clones," *Nature*, 312, 330–336 (Nov. 22, 1984).

Woods et al., "Isolation of a cDNA Clone Corresponding to the MHC Liked Complement Protein Factor B," *Mol. Immunology*, 19, 1411 (1982).

Woods et al., "Isolation of cDNA clones for the human complement protein factor B, a class III major histocompatability complex gene product," *P.N.A.S. USA* 79, 5661–5665 (Sep. 1982).

Woods et al., "Isolation of Class III cDNA Clones," Second Meeting on Cloning of the HLA and H–2 Regions, Abstract (Apr. 17–19, 1983).

Yanagawa et al., "Hybridomas for Production of Monoclonal antibodies to Human Erythropoetin," *Blood*, 64(2), 357–364 (Aug. 1984).

Yanagawa et al., "Isolation of Human Erythropoietin with Monoclonal Antibodies," *J. Biol. Chem.*, 259(5), 2707–2710 (Mar. 10, 1984).

Yanagi, "Recombinant Human Erythropoietin Produced by Namalwa Cells," *DNA*, 8(6), 419–427 (1989).

Young et al., "Efficient isolation of genes by using antibody probes," *P.N.A.S.* 80, 1194–1198 (Mar. 1983).

Yuen et al., "The Spectrum of N–linked oligosaccharide structures detected by enzymic microsequencing on a recombinant soluble CD4 glycoprotein from Chinese hamster ovary cells," *Eur. J. Biochem.*, 192, 523–528 (1990).

Zinn et al., "Regulated expression of an extrachromosomal human β–interferon gene in mouse cells,"*P.N.A.S.* (*USA*), 79, 4897–4901 (Aug. 1982).

Goldwasser, "Erythropoietin" pp. 1091–1103 (at p. 1092) in *Peptide Hormones*, (Bersen et al., North Holand Publishing Co., Amsterdam, 1973).

Miyake et al. 1977. Journal Biol. Chem. 252(15): 5558–5564.

FIG. 1

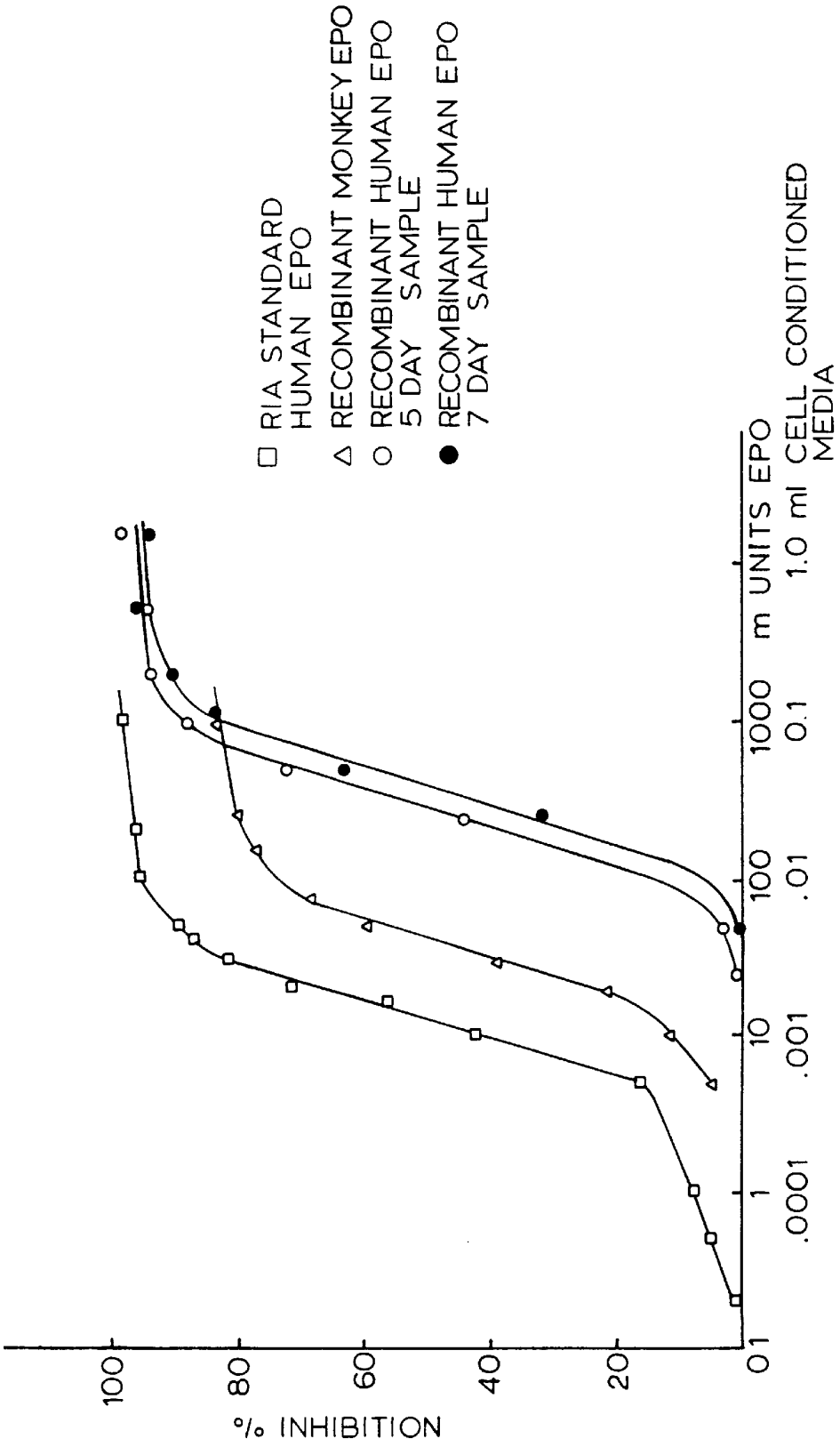

□ RIA STANDARD HUMAN EPO
△ RECOMBINANT MONKEY EPO
○ RECOMBINANT HUMAN EPO 5 DAY SAMPLE
● RECOMBINANT HUMAN EPO 7 DAY SAMPLE
% INHIBITION
100
80
60
40
20
0
.0001
.001
.01
0.1
1
10
100
1000
m UNITS EPO
1.0 ml CELL CONDITIONED MEDIA

FIG. 2

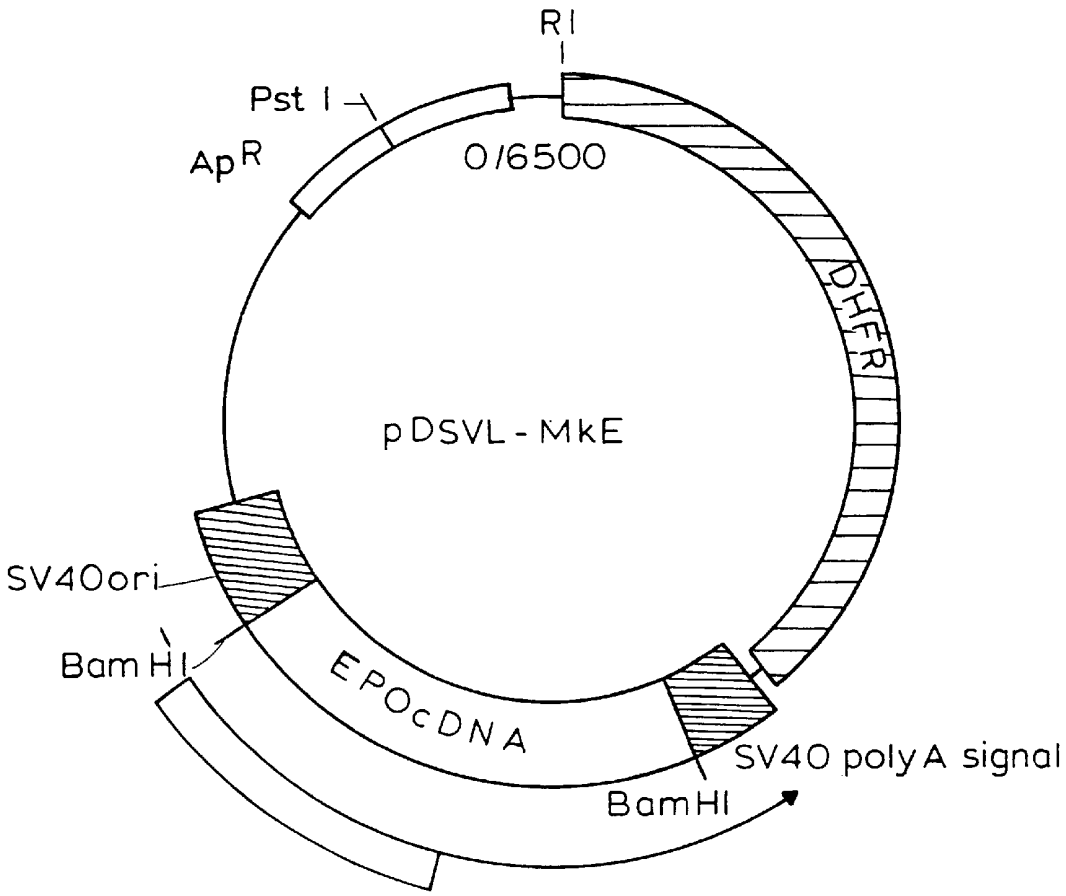

RI
Pst I
ApR
0/6500
DHFR
pDSVL - MkE
SV40ori
BamHI
EPOcDNA
SV40 polyA signal
BamHI

FIG. 3

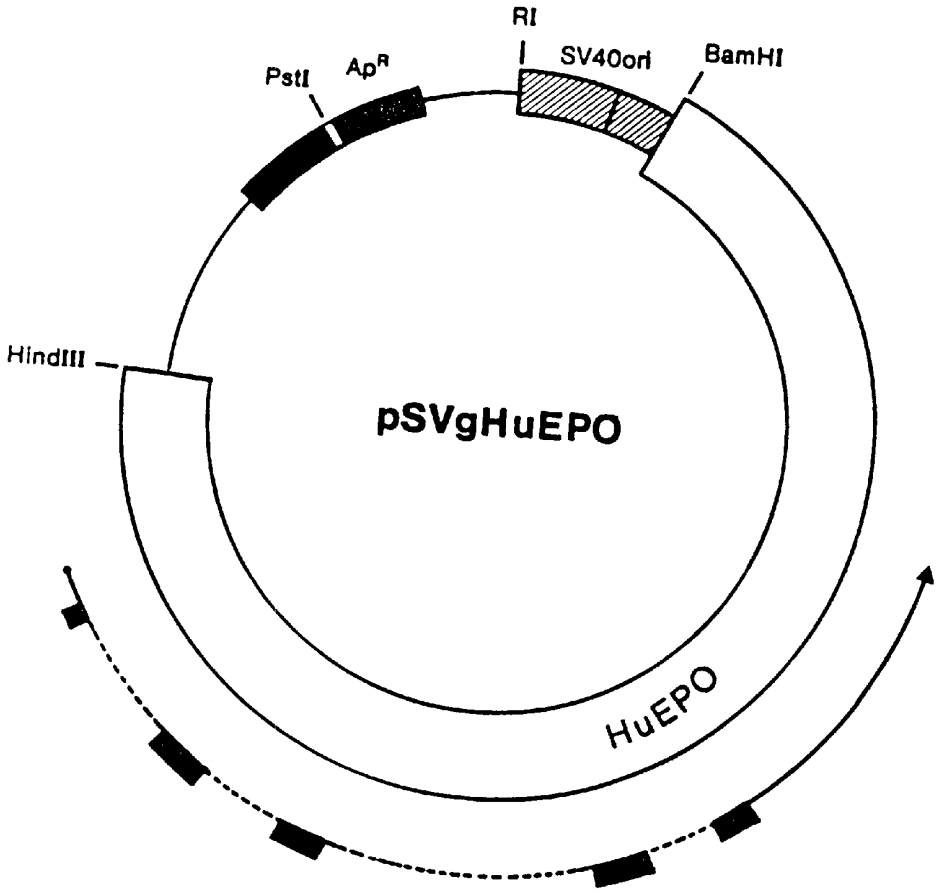


FIG. 4

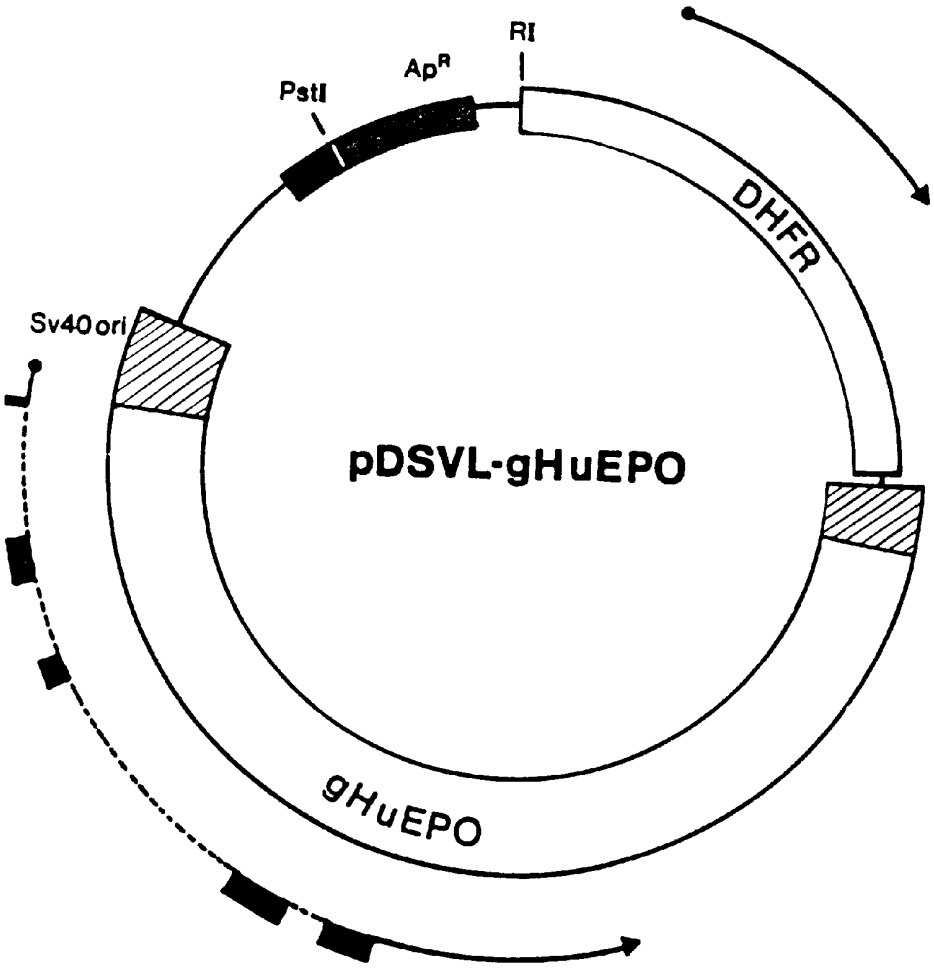


FIG. 5A

Sau3A

```
GATCCCGCGCCCCCTGGACAGCCGCCCTCTCCTCCAGGCCCGTGGGGCTGGCCCTGCCC

CGCTGAACTTCCCGGGATGAGGACTCCCGGTGTGGTCACCGCGCGCCTAGGTCGCTGAG

                        -27                             -20
                        Met Gly Val His Glu Cys Pro Ala Trp
GGACCCCGGCCAGGCGCGGAGATG GGG GTG CAC GAA TGT CCT GCC TGG

                                    -10
Leu Trp Leu Leu Leu Ser Leu Val Ser Leu Pro Leu Gly Leu Pro
CTG TGG CTT CTC CTG TCT CTC GTG TCG CTC CCT CTG GGC CTC CCA

          -1  +1                                    10
Val Pro Gly Ala Pro Pro Arg Leu Ile Cys Asp Ser Arg Val Leu
GTC CCG GGC GCC CCA CCA CGC CTC ATC TGT GAC AGC CGA GTC CTG

                                20                  *
Glu Arg Tyr Leu Leu Glu Ala Lys Glu Ala Glu Asn Val Thr Met
GAG AGG TAC CTC TTG GAG GCC AAG GAG GCC GAG AAT GTC ACG ATG

          30                                    *       40
Gly Cys Ser Glu Ser Cys Ser Leu Asn Glu Asn Ile Thr Val Pro
GGC TGT TCC GAA AGC TGC AGC TTG AAT GAG AAT ATC ACC GTC CCA
```

FIG.5B

```
                                    50
Asp Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg Met Glu Val Gly
GAC ACC AAA GTT AAC TTC TAT GCC TGG AAG AGG ATG GAG GTC GGG

                60                  70
Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu Leu Ser Glu
CAG CAG GCT GTA GAA GTC TGG CAG GGC CTG GCC CTG CTC TCA GAA

                                    *
Ala Val Leu Arg Gly Gln Ala Val Leu Ala Asn Ser Ser Gln Pro
GCT GTC CTG CGG GGC CAG GCC GTG TTG GCC AAC TCT TCC CAG CCT

        90                  100
Phe Glu Pro Leu Gln Leu His Met Asp Lys Ala Ile Ser Gly Leu
TTC GAG CCC CTG CAG CTG CAC ATG GAT AAA GCC ATC AGT GGC CTT

                110                 120
Arg Ser Ile Thr Leu Leu Arg Ala Leu Gly Ala Gln Glu Ala
CGC AGC ATC ACC ACT CTG CTT CGG GCG CTG GGA GCC CAG GAA GCC

        130                 140
Ile Ser Leu Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg Thr Ile
ATC TCC CTC CCA GAT GCG GCC TCG GCT GCT CCA CTC CGA ACC ATC

                                    150
Thr Ala Asp Thr Phe Cys Lys Leu Phe Arg Val Tyr Ser Asn Phe
ACT GCT GAC ACT TTC TGC AAA CTC TTC CGA GTC TAC TCC AAT TTC
```

FIG. 5C

```
        150                                                 160
Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys Arg Arg
CTC CGG GGA AAG CTG AAG CTG TAC ACG GGG GAG GCC TGC AGG AGA

        165
Gly Asp Arg OP
GGG GAC AGA TGA CCAGGTGCGTCCAGCTGGGCACATCCACCACCTCCCTCACCAACA

CTGCCTGTGCCACACCCTCCCTCACCACTCCCGAACCCCATCGAGGGGCTCTCAGCTAAG

CGCCAGCCTGTCCCATGGACACTCCAGTGCCAGCAATGACATCTCAGGGGCCAGAGGAAC

TGTCCAGAGCACAACTCTGAGATCTAAGGATGTCGCAGGGCCAACTTGAGGGCCCAGAGC

AGGAAGCATTCAGAGAGCAGCTTTAAACTCAGGAGCAGAGACAATGCAGGGAAAACACCT

GAGCTCACTCGGCCACCTGCAAAATTTGATGCAGGACACGCTTTGGAGGCAATTTACCTG

TTTTTGCACCTACCATCAGGGACAGGATGACTGGAGAACTTAGGTGGCAAGCTGTGACTT

CTCAAGGCCTCACGGGCACTCCCTTGGTGGCAAGAGCCCCCTTGACACTGAGAGAATATT

TTGCAATCTGCAGCAGGAAAAATTACGGACAGGTTTTGGAGGTTGGAGGGTACTTGACAG

GTGTGTGGGGAAGCAGGGCGGTAGGGGTGGAGCTGGGATGCGAGTGAGAACCGTGAAGAC

AGGATGGGGGCTGGCCTCTGGTTCTCGTGGGGTCCAAGCTT
                                      HindIII
```

FIG.6A

```
AAGCTTCTGGGCTTCCAGACCCAGCTACTTTGCGGAACTCAGCAACCCAGGCATCTCTGAGTCTCCGCCCA

AGACCGGGATGCCCCCCCAGGGGAGGTGTCCGGGAGCCCAGCCTTTCCCAGATAGCACGCTCCGCCAGTCCC

AAGGGTGCGCAACCGGCTGCACTCCCCTCCCGCGACCCAGGGCCCGGGAGCAGCCCCCATGACCCACACGC

ACGTCTGCAGCAGCCCCCGCTCACGCCCCGGCGAGCCTCAACCCAGGCGTCCTGCCCCTGCTCTGACCCCGG

GTGGCCCCTACCCCCTGGCGACCCCCTCACGCACACAGCCTCTCCCCCCACCCCCGCGCACGCACACATG

CAGATAACAGCCCCCGACCCCCCGGCCAGAGCCGXAGAGTCCCTGGGCCACCCCGGCCGCTCGCCTGCCTG

CGCCGCACCGCGCTGTCCTCCCGGAGCCGGACCGGGGCCACCGCGCCCXGCTCTGCTCCGACACCGCGCCC

CTTGGACAGCCGCCCTCTCCTCTAGGCCCGTGGGGCTGGCCCTGCACCGCCGAGCTTCCCGGGATGAGGXX

                                    -27        -24
                                    Met Gly Val His
CCCGGTGACCGGCGCGCCCCAAGTCGCTGAGGGACCCCGGCCAAGCGCGGAG  ATG GGG GTG CAC G

GTGAGTACTCGCGGGGCTGGGCGCTCCCGGCGGCCGGGTTCCTGTTTGAGCGGGGGATTTAGCGCCCCGGCT
```

FIG.6B

```
ATTGGCCAAGAGGTGGCTGGGTTCAAGGACCGGCGACTTGTCAAGGACCCCGGAAGGGGGAGGGGGGTGGG

GCAGCCTCCACGTGCCGCGGGGACTTGGGGGAGTTCTTGGGGATGGCAAAAACCTGGCCTGTTGAGGGGCA

CAGTTTGGGGTTGGGGAGGAGGTTTGGGGTTCTGCTGTGCAGTTGTGTCGTTGTCAGTGTCTCG[I·S·]

TTGCACACGCACAGATCAATAAGCCAGAGGCAGCACCTGAGTGCTTGCATGGTTGGGACAGGAAGGACGAG

CTGGGGCAGAGACGTGGGGATGAAGGAAGCTGTCCTTCCACAGCCACCCTTCTCCCCCCCCGCCTGACTCT

                          -23          -20
                          Glu Cys Pro Ala Trp Leu Trp Leu Leu Leu Ser Leu
CAGCCTGGCTATCTGTTCTAG      AA TGT CCT GCC TGG CTG TGG CTT CTC CTG TCC CTG

    -10                                    -1  +1
Leu Ser Leu Pro Leu Gly Leu Pro Val Leu Gly Ala Pro Pro Arg Leu Ile Cys
CTG TCG CTC CCT CTG GGC CTC CCA GTC CTG GGC GCC CCA CCA CGC CTC ATC TGT

         10                                        20                *
Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala Lys Glu Ala Glu Asn Ile
GAC AGC CGA GTC CTG GAG AGG TAC CTC TTG GAG GCC AAG GAG GCC GAG AAT ATC

 26
Thr
ACG GTGAGACCCCTTCCCCAGCACATTCCACAGAACTCACGCTCAGGGCTTCAGGGAACTCCTCCCAGAT

CCAGGAACCTGGCACTTGGTTTGGGGTGGAGTTGGGAAGCTAGACACTGCCCCCCTACATAAGAATAAGTC
```

FIG.6C

```
TGGTGGCCCCAAACCATACCTGAAACTAGGCAAGGAGCAAAGCCAGCAGATCCTACGCCTGTGGGCCAGGG

                                  27                  30
                                 Thr Gly Cys Ala Glu
CCAGAGCCTTCAGGGACCCTTGACTCCCCGGGCTGTGTGCATTTCAG    ACG GGC TGT GCT GAA

                          *       40
His Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr
CAC TGC AGC TTG AAT GAG AAT ATC ACT GTC CCA GAC ACC AAA GTT AAT TTC TAT

50                  55
Ala Trp Lys Arg Met Glu
GCC TGG AAG AGG ATG GAG GTGAGTTCCTTTTTTTTTTTTTCCTTTCTTTTGGAGAATCTCATT

TGCGAGCCTGATTTTGGATGAAAGGGAGAATGATCGGGGGAAAGGTAAAATGGAGCAGCAGAGATGAGGCT

GCCTGGGCGCAGAGGCTCACGTCTATAATCCCAGGCTGAGATGGCCGAGATGGGAGAATTGCTTGAGCCCT

GGAGTTTCAGACCAACCTAGGCAGCATAGTGAGATCCCCCCATCTCTACAAACATTTAAAAAAATTAGTCAG

GTGAAGTGGTGCATGGTGGTAGTCCCAGATATTTGGAAGGCTGAGGCGGGAGGATCGCTTGAGCCCAGGAA

TTTGAGGCTGCAGTGAGCTGTGATCACACCACTGCACTCCAGCCTCAGTGACAGAGTGAGGCCCTGTCTCA
```

FIG. 6D

```
AAAAAGAAAAGAAAAAAGAAAAATAATGAGGGCTGTATGGAATACATTCATTATTCATTCACTCACTCACT

CACTCATTCATTCATTCATTCATTCAACAAGTCTTATTGCATACCTTCTGTTTGCTCAGCTTGGTGCTTGG

GGCTGCTGAGGGGCAGGAGGGAGAGGGTGACATGGGTCAGCTCGACTCCCAGAGTCCACTCCCTGTAG

 56                60                                                  70
Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu Leu Ser Glu Ala
GTC GGG CAG CAG GCC GTA GAA GTC TGG CAG GGC CTG GCC CTG CTG TCG GAA GCT

                           80           *                              90
Val Leu Arg Gly Gln Ala Leu Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu
GTC CTG CGG GGC CAG GCC CTG TTG GTC AAC TCT TCC CAG CCG TGG GAG CCC CTG

                                  100
Gln Leu His Val Asp Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu
CAG CTG CAT GTG GAT AAA GCC GTC AGT GGC CTT CGC AGC CTC ACC ACT CTG CTT

110                 115
Arg Ala Leu Gly Ala Gln
CGG GCT CTG GGA GCC CAG GTGAGTAGGAGCGGACACTTCTGCTTGCCCTTTCTGTAAGAAGGGGA

GAAGGGTCTTGCTAAGGAGTACAGGAACTGTCCGTATTCCTTCCCTTTCTGTGGCACTGCAGCGACCTCCT

                    116             120
                    Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala
GTTTTCTCCTTGGCAG    AAG GAA GCC ATC TCC CCT CCA GAT GCG GCC TCA GCT GCT
```

FIG. 6E

```
                                                    130
Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val Tyr Ser
CCA CTC CGA ACA ATC ACT GCT GAC ACT TTC CGC AAA CTC TTC CGA GTC TAC TCC

                                                    140
Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys Arg Thr Gly
AAT TTC CTC CGG GGA AAG CTG AAG CTG TAC ACA GGG GAG GCC TGC AGG ACA GGG

                                                    150
Asp Arg 0P                                          160
GAC AGA TGA CCAGGTGTGTCCACCTGGGCATATCCACCACCTCCCTCACCAACATTGCTTGTGCCACA

CCCTCCCCCGCCACTCCTGAACCCCGTCGAGGGGCTCTCAGCTCAGCGCCAGCCTGTCCCATGGACACTCC

AGTGCCAGCAATGACATCTCAGGGGCCAGAGGAACTGTCCAGAGAGCAACTCTGAGATCTAAGGATGTCAC

AGGGCCAACTTGAAGGGCCCAGAGCAGGAAGCATTCAGAGAGCAGCTTTAAACTCAGGGACAGAGCCATGC

TGGGAAGACGCGCCTGAGCTCACTCGGCACCCTGCAAAATTTGATGCCAGGACACGCTTTGGAGGCGATTTAC

CTGTTTTCGCACCTACCATCAGGGACAGGATGACCTGGAGAACTTAGGTGGCAAGCTGTGACTTCTCCAGG

TCTCACGGGCATGGGCACTCCCTTGGTGGCAAGAGCCCCCTTGACACCGGGGTGGTGGGAACCATGAAGAC

AXGATXGGGGCTGGCCTCTCATGGGGTCCAAGTTTTGTGTATTCTCAACCTATTGACAGACTGAA

ACACAATATGAC
```

FIG. 7

```
                                        -1  1
          XbaI                          MetAla
     CTAG AAACCATGAG GGTAATAAAA TAATGGCTCC GCCGCGTCTG
          TTTGGTACTC CCATTATTTT ATTACCGAGG CGGCGCAGAC

ATCTGCGACT CGAGAGTTCT GGAACGTTAC CTGCTGGAAG CTAAAGAAGC
TAGACGCTGA GCTCTCAAGA CCTTGCAATG GACGACCTTC GATTTCTTCG

TGAAAACATC ACCACTGGTT GTGCTGAACA CTGTTCTTTG AACGAAAACA
ACTTTTGTAG TGGTGACCAA CACGACTTGT GACAAGAAAC TTGCTTTTGT

TTACGGTACC AGACACCAAG GTTAACTTCT ACGCTTGGAA ACGTATGGAA
AATGCCATGG TCTGTGGTTC CAATTGAAGA TGCGAACCTT TGCATACCTT

GTTGGTCAAC AAGCAGTTGA AGTTTGGCAG GGTCTGGCAC TGCTGAGCGA
CAACCAGTTG TTCGTCAACT TCAAACCGTC CCAGACCGTG ACGACTCGCT

GGCTGTACTG CGTGGCCAGG CACTGCTGGT AAACTCCTCT CAGCCGTGGG
CCGACATGAC GCACCGGTCC GTGACGACCA TTTGAGGAGA GTCGGCACCC

AACCGCTGCA GCTGCATGTT GACAAAGCAG TATCTGGCCT GAGATCTCTG
TTGGCGACGT CGACGTACAA CTGTTTCGTC ATAGACCGGA CTCTAGAGAC

ACTACTCTGC TGCGTGCTCT GGGTGCACAG AAAGAGGCTA TCTCTCCGCC
TGATGAGACG ACGCACGAGA CCCACGTGTC TTTCTCCGAT AGAGAGGCGG

GGATGCTGCA TCTGCTGCAC CGCTGCGTAC CATCACTGCT GATACCTTCC
CCTACGACGT AGACGACGTG GCGACGCATG GTAGTGACGA CTATGGAAGG

GCAAACTGTT TCGTGTATAC TCTAACTTCC TGCGTGGTAA ACTGAAACTG
CGTTTGACAA AGCACATATG AGATTGAAGG ACGCACCATT TGACTTTGAC

                                              SalI
TATACTGGCG AAGCATGCCG TACTGGTGAC CGCTAATAG
ATATGACCGC TTCGTACGGC ATGACCACTG GCGATTATCA GCT
```

FIG. 8

```
                -1 +1
HindIII         ArgAla
AGCTTGGATA AAAGAGCTCC ACCAAGATTG ATCTGTGACT CGAGAGTTTT
    ACCTAT TTTCTCGAGG TGGTTCTAAC TAGACACTGA GCTCTCAAAA

GGAAAGATAC TTGTTGGAAG CTAAAGAAGC TGAAAACATC ACCACTGGTT
CCTTTCTATG AACAACCTTC GATTTCTTCG ACTTTTGTAG TGGTGACCAA

GTGCTGAACA CTGTTCTTTG AACGAAAACA TTACGGTACC AGACACCAAG
CACGACTTGT GACAAGAAAC TTGCTTTTGT AATGCCATGG TCTGTGGTTC

GTTAACTTCT ACGCTTGGAA ACGTATGGAA GTTGGTCAAC AAGCTGTTGA
CAATTGAAGA TGCGAACCTT TGCATACCTT CAACCAGTTG TTCGACAACT

AGTTTGGCAA GGTTTGGCCT TGTTATCTGA AGCTGTTTTG AGAGGTCAAG
TCAAACCGTT CCAAACCGGA ACAATAGACT TCGACAAAAC TCTCCAGTTC

CCTTGTTGGT TAACTCTTCT CAACCATGGG AACCATTGCA ATTGCACGTC
GGAACAACCA ATTGAGAAGA GTTGGTACCC TTGGTAACGT TAACGTGCAG

GATAAAGCCG TCTCTGGTTT GAGATCTTTG ACTACTTTGT TGAGAGCTTT
CTATTTCGGC AGAGACCAAA CTCTAGAAAC TGATGAAACA ACTCTCGAAA

GGGTGCTCAA AAGGAAGCCA TTTCCCCACC AGACGCTGCT TCTGCCGCTC
CCCACGAGTT TTCCTTCGGT AAAGGGGTGG TCTGCGACGA AGACGGCGAG

CATTGAGAAC CATCACTGCT GATACCTTCA GAAAGTTATT CAGAGTTTAC
GTAACTCTTG GTAGTGACGA CTATGGAAGT CTTTCAATAA GTCTCAAATG

TCCAACTTCT TGAGAGGTAA ATTGAAGTTG TACACCGGTG AAGCCTGTAG
AGGTTGAAGA ACTCTCCATT TAACTTCAAC ATGTGGCCAC TTCGGACATC

AACTGGTGAC AGATAAGCCC GACTGATAAC AACAGTGTAG
TTGACCACTG TCTATTCGGG CTGACTATTG TTGTCACATC

             SalI
ATGTAACAAA G
TACATTGTTT CAGCT
```

FIG. 9

```
                -20       -10       +1        10        20        30        40
Human   MGVHECPAWLWLLSLLSLPLGLPVLGAPPRLICDSRVLERYLLEAKEAENITTGCAEHCSLNENITVPDTK
        ********************* ********************** * ** * ********************
Monkey  MGVHECPAWLWLLSLVSLPLGLPVPGAPPRLICDSRVLERYLLEAKEAENVTMGCSESCSLNENITVPDTK

            50        60        70        80        90        100       110
Human   VNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVDKAVSGLRSLTTLLRALGAQKE
        ********************************** * ****** ****** *** ***** ********** *
Monkey  VNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQAVLANSSQPFEPLQLHMDKAISGLRSITTLLRALGAQ-E

            120       130       140       150       160
Human   AISPPDAASAAPLRTITADTFRKLFRVYSNFLRGKLKLYTGEACRTGDR
        *** ********************** ********************** ***
Monkey  AISLPDAASAAPLRTITADTFCKLFRVYSNFLRGKLKLYTGEACRRGDR
```

FIG. 10

1. AATTCTAGAAACCATGAGGGTAATAAAATA

2. CCATTATTTTATTACCCTCATGGTTTCTAG

3. ATGGCTCCGCCGCGTCTGATCTGCGAC

4. CTCGAGTCGCAGATCAGACGCGGCGGAG

5. TCGAGAGTTCTGGAACGTTACCTGCTG

6. CTTCCAGCAGGTAACGTTCCAGAACT

7. GAAGCTAAAGAAGCTGAAAACATC

8. GTGGTGATGTTTTCAGCTTCTTTAG

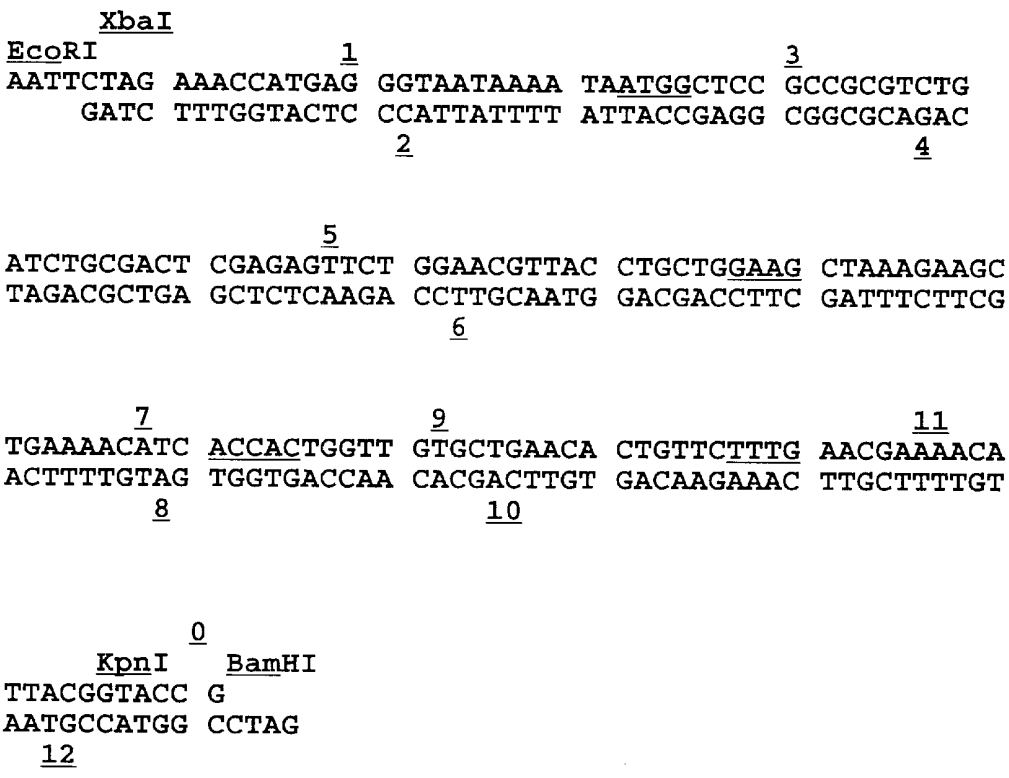
9. ACCACTGGTTGTGCTGAACACTGTTC

10. CAAAGAACAGTGTTCAGCACAACCA

11. TTTGAACGAAAACATTACGGTACCG

12. GATCCGGTACCGTAATGTTTTCGTT

FIG. 11


XbaI
EcoRI
1
3
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GATC TTTGGTACTC CCATTATTTT ATTACCGAGG CGGCGCAGAC
2
4
5
ATCTGCGACT CGAGAGTTCT GGAACGTTAC CTGCTGGAAG CTAAAGAAGC
TAGACGCTGA GCTCTCAAGA CCTTGCAATG GACGACCTTC GATTTCTTCG
6
7
9
11
TGAAAACATC ACCACTGGTT GTGCTGAACA CTGTTCTTTG AACGAAAACA
ACTTTTGTAG TGGTGACCAA CACGACTTGT GACAAGAAAC TTGCTTTTGT
8
10
0
KpnI
BamHI
TTACGGTACC G
AATGCCATGG CCTAG
12

FIG. 12

1. AATTCGGTACCAGACACCAAGGT

2. GTTAACCTTGGTGTCTGGTACCG

3. TAACTTCTACGCTTGGAAACGTAT

4. TTCCATACGTTTCCAAGCGTAGAA

5. GGAAGTTGGTCAACAAGCAGTTGAAGT

6. CCAAACTTCAACTGCTTGTTGACCAAC

7. TTGGCAGGGTCTGGCACTGCTGAGCG

8. GCCTCGCTCAGCAGTGCCAGACCCTG

9. AGGCTGTACTGCGTGGCCAGGCA

10. GCAGTGCCTGGCCACGCAGTACA

11. CTGCTGGTAAACTCCTCTCAGCCGT

12. TTCCCACGGCTGAGAGGAGTTTACCA

13. GGGAACCGCTGCAGCTGCATGTTGAC

14. GCTTTGTCAACATGCAGCTGCAGCGG

15. AAAGCAGTATCTGGCCTGAGATCTG

16. GATCCAGATCTCAGGCCAGATACT

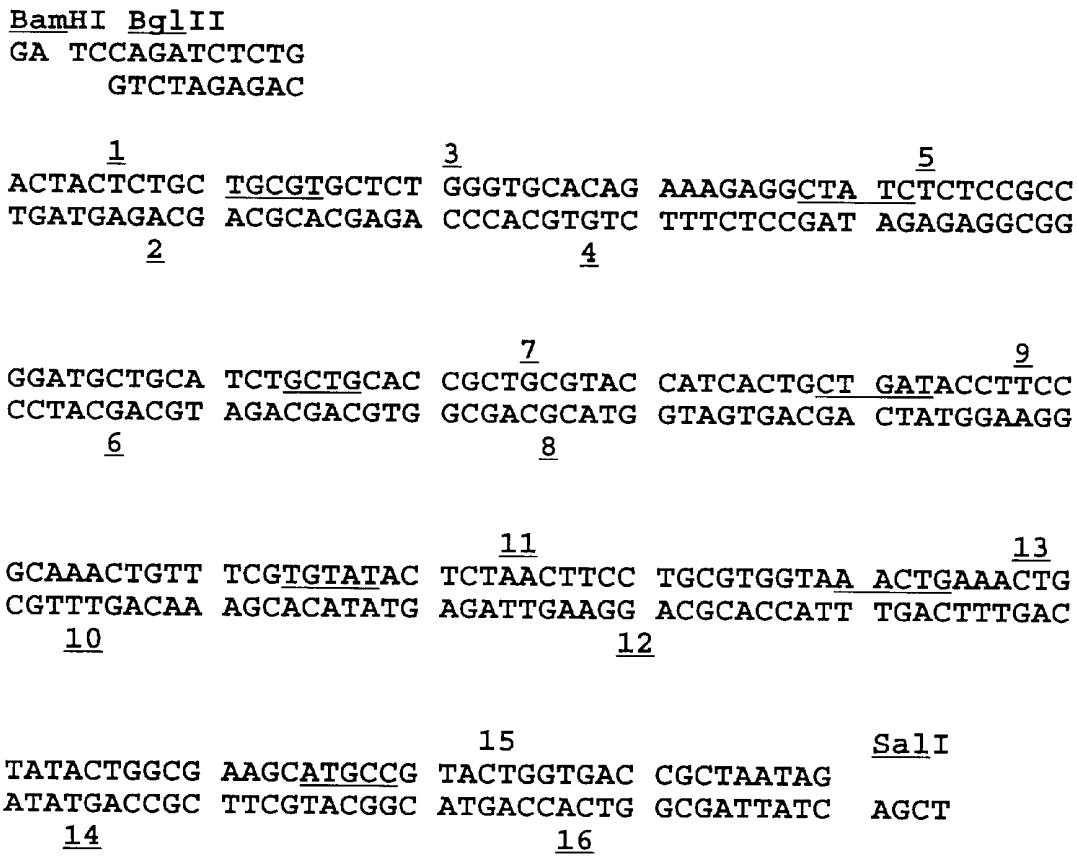
FIG. 13

EcoRI KpnI
1
A ATTCGGTACC AGACACCAAG GTTAACTTCT ACGCTTGGAA ACGTATGGAA
GCCATGG TCTGTGGTTC CAATTGAAGA TGCGAACCTT TGCATACCTT
2
3
4
5
GTTGGTCAAC AAGCAGTTGA AGTTTGGCAG GGTCTGGCAC TGCTGAGCGA
CAACCAGTTG TTCGTCAACT TCAAACCGTC CCAGACCGTG ACGACTCGCT
6
7
8
9
GGCTGTACTG CGTGGCCAGG CACTGCTGGT AAACTCCTCT CAGCCGTGGG
CCGACATGAC GCACCGGTCC GTGACGACCA TTTGAGGAGA GTCGGCACCC
10
11
12
13
AACCGCTGCA GCTGCATGTT GACAAAGCAG TATCTGGCCT GAGATCTG
TTGGCGACGT CGACGTACAA CTGTTTCGTC ATAGACCGGA CTCTAGACCTAC
14
15
16
BglIII BamHI

FIG. 14

1. GATCCAGATCTCTGACTACTCTGC
2. ACGCAGCAGAGTAGTCAGAGATCTG
3. TGCGTGCTCTGGGTGCACAGAAAGAGG
4. GATAGCCTCTTTCTGTGCACCCAGAGC
5. CTATCTCTCCGCCGGATGCTGCATCT
6. CAGCAGATGCAGCATCCGGCGGAGA
7. GCTGCACCGCTGCGTACCATCACTG
8. ATCAGCAGTGATGGTACGCAGCGGTG
9. CTGATACCTTCCGCAAACTGTTTCG
10. ATACACGAAACAGTTTGCGGAAGGT
11. TGTATACTCTAACTTCCTGCGTGGTA
12. CAGTTTACCACGCAGGAAGTTAGAGT
13. AACTGAAACTGTATACTGGCGAAGC
14. GGCATGCTTCGCCAGTATACAGTTT
15. ATGCCGTACTGGTGACCGCTAATAG
16. TCGACTATTAGCGGTCACCAGTAC

FIG. 15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GTCTAGAGAC
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3
5
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TGATGAGACG ACGCACGAGA CCCACGTGTC TTTCTCCGAT AGAGAGGCGG
2
4
7
9
GGATGCTGCA TCTGCTGCAC CGCTGCGTAC CATCACTGCT GATACCTTCC
CCTACGACGT AGACGACGTG GCGACGCATG GTAGTGACGA CTATGGAAGG
6
8
11
13
GCAAACTGTT TCGTGTATAC TCTAACTTCC TGCGTGGTAA ACTGAAACTG
CGTTTGACAA AGCACATATG AGATTGAAGG ACGCACCATT TGACTTTGAC
10
12
15
SalI
TATACTGGCG AAGCATGCCG TACTGGTGAC CGCTAATAG
ATATGACCGC TTCGTACGGC ATGACCACTG GCGATTATC AGCT
14
16

FIG. 16

1. AATTCAAGCTTGGATAAAAGAGCT

2. GTGGAGCTCTTTTATCCAAGCTTG

3. CCACCAAGATTGATCTGTGACTC

4. TCTCGAGTCACAGATCAATCTTG

5. GAGAGTTTTGGAAAGATACTTGTTG

6. CTTCCAACAAGTATCTTTCCAAAAC

7. GAAGCTAAAGAAGCTGAAAACATC

8. GTGGTGATGTTTTCAGCTTCTTTAG

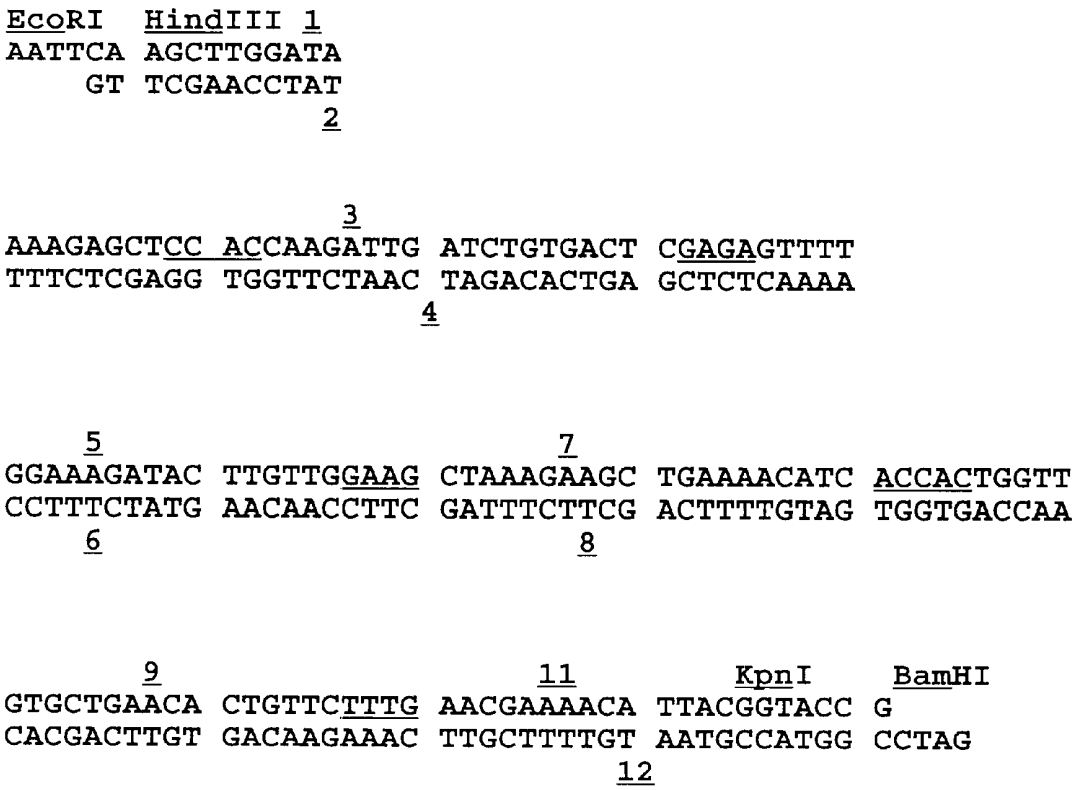
9. ACCACTGGTTGTGCTGAACACTGTTC

10. CAAAGAACAGTGTTCAGCACAACCA

11. TTTGAACGAAAACATTACGGTACCG

12. GATCCGGTACCGTAATGTTTTCGTT

FIG. 17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3
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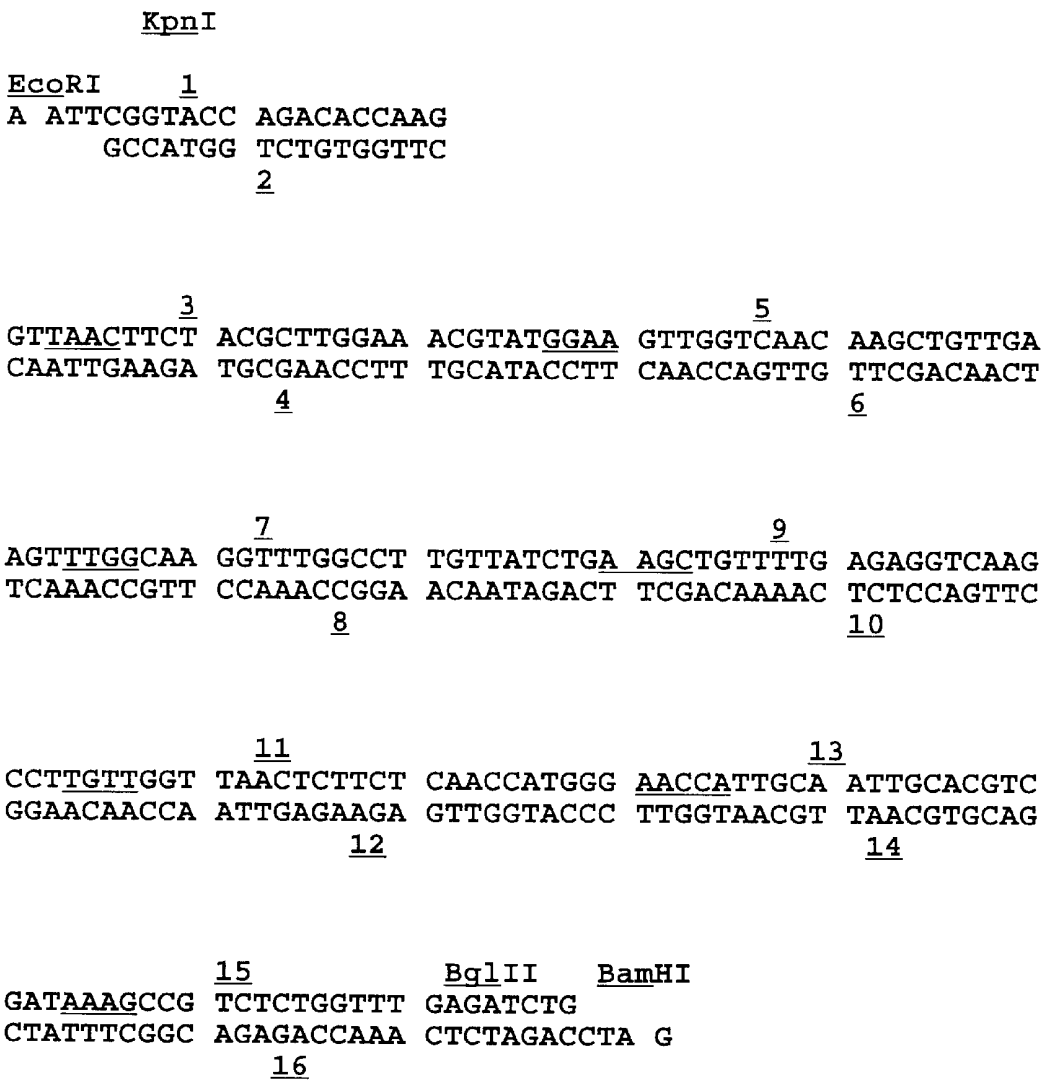
4
5
7
GGAAAGATAC TTGTTGGAAG CTAAAGAAGC TGAAAACATC ACCACTGGTT
CCTTTCTATG AACAACCTTC GATTTCTTCG ACTTTTGTAG TGGTGACCAA
6
8
9
11
KpnI
BamHI
GTGCTGAACA CTGTTCTTTG AACGAAAACA TTACGGTACC G
CACGACTTGT GACAAGAAAC TTGCTTTTGT AATGCCATGG CCTAG
12

FIG. 18

1. AATTCGGTACCAGACACCAAGGT
2. GTTAACCTTGGTGTCTGGTACCG
3. TAACTTCTACGCTTGGAAACGTAT
4. TTCCATACGTTTCCAAGCGTAGAA
5. GGAAGTTGGTCAACAAGCAGTTGAAGT
6. CCAAACTTCAACTGCTTGTTGACCAAC
7. TTGGCAAGGTTTGGCCTTGTTATCTG
8. GCTTCAGATAACAAGGCCAAACCTTG
9. AAGCTGTTTTGAGAGGTGAAGCCT
10. AACAAGGCTTGACCTCTCAAAACA
11. TGTTGGTTAACTCTTCTCAACCATGGG
12. TGGTTCCCATGGTTGAGAAGAGTTAACC
13. AACCATTGCAATTGCACGTCGAT
14. CTTTATCGACGTGCAATTGCAA
15. AAAGCCGTCTCTGGTTTGAGATCTG
16. GATCCAGATCTCAAACCAGAGACGG

FIG. 19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EcoRI
1
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GCCATGG TCTGTGGTTC
2
3
5
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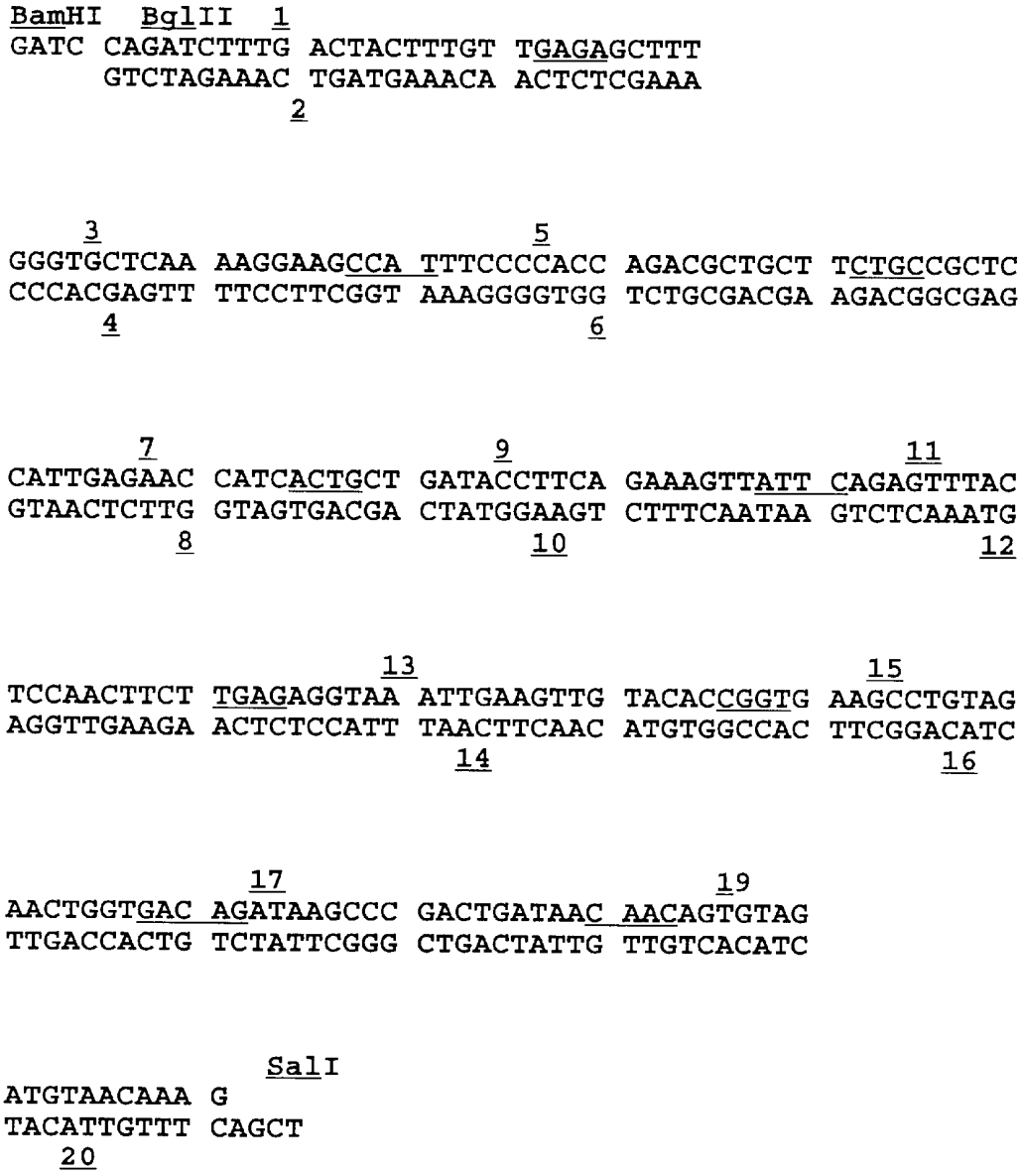
CAATTGAAGA TGCGAACCTT TGCATACCTT CAACCAGTTG TTCGACAACT
4
6
7
9
AGTTTGGCAA GGTTTGGCCT TGTTATCTGA AGCTGTTTTG AGAGGTCAAG
TCAAACCGTT CCAAACCGGA ACAATAGACT TCGACAAAAC TCTCCAGTTC
8
10
11
13
CCTTGTTGGT TAACTCTTCT CAACCATGGG AACCATTGCA ATTGCACGTC
GGAACAACCA ATTGAGAAGA GTTGGTACCC TTGGTAACGT TAACGTGCAG
12
14
15
BglII
BamHI
GATAAAGCCG TCTCTGGTTT GAGATCTG
CTATTTCGGC AGAGACCAAA CTCTAGACCTA G
16

FIG. 20

1. GATCCAGATCTTTGACTACTTTGTT
2. TCTCAACAAAGTAGTCAAAGATCTG
3. GAGAGCTTTGGGTGCTCAAAAGGAAG
4. ATGGCTTCCTTTTGAGCACCCAAAGC
5. CCATTTCCCCACCAGACGCTGCTT
6. GCAGAAGCAGCGTCTGGTGGGGAA
7. CTGCCGCTCCATTGAGAACCATC
8. CAGTGATGGTTCTCAATGGAGCG
9. ACTGCTGATACCTTCAGAAAGTT
10. GAATAACTTTCTGAAGGTATCAG
11. ATTCAGAGTTTACTCCAACTTCT
12. CTCAAGAAGTTGGAGTAAACTCT
13. TGAGAGGTAAATTGAAGTTGTACAC
14. ACCGGTGTACAACTTCAATTTACCT
15. CGGTGAAGCCTGTAGAACTGGT
16. CTGTCACCAGTTCTACAGGCTTC
17. GACAGATAAGCCCGACTGATAA
18. GTTGTTATCAGTCGGGCTTAT
19. CAACAGTGTAGATGTAACAAAG
20. TCGACTTTGTTACATCTACACT

FIG. 21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3
5
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CCCACGAGTT TTCCTTCGGT AAAGGGGTGG TCTGCGACGA AGACGGCGAG
4
6
7
9
11
CATTGAGAAC CATCACTGCT GATACCTTCA GAAAGTTATT CAGAGTTTAC
GTAACTCTTG GTAGTGACGA CTATGGAAGT CTTTCAATAA GTCTCAAATG
8
10
12
13
15
TCCAACTTCT TGAGAGGTAA ATTGAAGTTG TACACCGGTG AAGCCTGTAG
AGGTTGAAGA ACTCTCCATT TAACTTCAAC ATGTGGCCAC TTCGGACATC
14
16
17
19
AACTGGTGAC AGATAAGCCC GACTGATAAC AACAGTGTAG
TTGACCACTG TCTATTCGGG CTGACTATTG TTGTCACATC
SalI
ATGTAACAAA G
TACATTGTTT CAGCT
20

PRODUCTION OF ERTHROPOIETIN

This is a continuation of application Ser. No. 07/957,073, filed Oct. 6, 1992, abandoned, which is a continuation of application Ser. No. 07/609,741, filed Nov. 6, 1990, now abandoned, which is a continuation of application Ser. No. 07/113,179, filed Oct. 23, 1987, now U.S. Pat. No. 5,441,868, which is a continuation of application Ser. No. 06/675,298, filed Nov. 30, 1984, now U.S. Pat. No. 4,703,008, which is a continuation in part of application Ser. No. 06/655,841, filed Sep. 28, 1984, now abandoned, which is a continuation in part of application Ser. No. 06/582,185, filed Feb. 21, 1984, now abandoned, which is a continuation in part of application Ser. No. 06/561,024, filed Dec. 13, 1983, now abandoned.

BACKGROUND

The present invention relates generally to the manipulation of genetic materials and, more particularly, to recombinant procedures making possible the production of polypeptides possessing part or all of the primary structural conformation and/or one or more of the biological properties of naturally-occurring erythropoietin.

A. Manipulation Of Genetic Materials

Genetic materials may be broadly defined as those chemical substances which program for and guide the manufacture of constituents of cells and viruses and direct the responses of cells and viruses. A long chain polymeric substance known as deoxyribonucleic acid (DNA) comprises the genetic material of all living cells and viruses except for certain viruses which are programmed by ribonucleic acids (RNA). The repeating units in DNA polymers are four different nucleotides, each of which consists of either a purine (adenine or guanine) or a pyrimidine (thymine or cytosine) bound to a deoxyribose sugar to which a phosphate group is attached. Attachment of nucleotides in linear polymeric form is by means of fusion of the 5' phosphate of one nucleotide to the 3 ' hydroxyl group of another. Functional DNA occurs in the form of stable double stranded associations of single strands of nucleotides (known as deoxyoligonucleotides), which associations occur by means of hydrogen bonding between purine and pyrimidine bases [i.e., "complementary" associations existing either between adenine (A) and thymine (T) or guanine (G) and cytosine (C)]. By convention, nucleotides are referred to by the names of their constituent purine or pyrimidine bases, and the complementary associations of nucleotides in double stranded DNA (i.e., A—T and G—C) are referred to as "base pairs". Ribonucleic acid is a polynucleotide comprising adenine, guanine, cytosine and uracil (U), rather than thymine, bound to ribose and a phosphate group.

Most briefly put, the programming function of DNA is generally effected through a process wherein specific DNA nucleotide sequences (genes) are "transcribed" into relatively unstable messenger RNA (mRNA) polymers. The mRNA, in turn, serves as a template for the formation of structural, regulatory and catalytic proteins from amino acids. This mRNA "translation" process involves the operations of small RNA strands (tRNA) which transport and align individual amino acids along the mRNA strand to allow for formation of polypeptides in proper amino acid sequences. The mRNA "message", derived from DNA and providing the basis for the tRNA supply and orientation of any given one of the twenty amino acids for polypeptide "expression", is in the form of triplet "codons"—sequential groupings of three nucleotide bases. In one sense, the formation of a protein is the ultimate form of "expression" of the programmed genetic message provided by the nucleotide sequence of a gene.

"Promoter" DNA sequences usually "precede" a gene in a DNA polymer and provide a site for initiation of the transcription into mRNA. "Regulator" DNA sequences, also usually "upstream" of (i.e., preceding) a gene in a given DNA polymer, bind proteins that determine the frequency (or rate) of transcriptional initiation. Collectively referred to as "promoter/regulator" or "control" DNA sequence, these sequences which precede a selected gene (or series of genes) in a functional DNA polymer cooperate to determine whether the transcription (and eventual expression) of a gene will occur. DNA sequences which "follow" a gene in a DNA polymer and provide a signal for termination of the transcription into mRNA are referred to as transcription "terminator" sequences.

A focus of microbiological processing for the last decade has been the attempt to manufacture industrially and pharmaceutically significant substances using organisms which either do not initially have genetically coded information concerning the desired product included in their DNA, or (in the case of mammalian cells in culture) do not ordinarily express a chromosomal gene at appreciable levels. Simply put, a gene that specifies the structure of a desired polypeptide product is either isolated from a "donor" organism or chemically synthesized and then stably introduced into another organism which is preferably a self-replicating unicellular organism such as bacteria, yeast or mammalian cells in culture. Once this is done, the existing machinery for gene expression in the "transformed" or "transfected" microbial host cells operates to construct the desired product, using the exogenous DNA as a template for transcription of mRNA which is then translated into a continuous sequence of amino acid residues.

The art is rich in patent and literature publications relating to "recombinant DNA" methodologies for the isolation, synthesis, purification and amplification of genetic materials for use in the transformation of selected host organisms. U.S. Pat. No. 4,237,224 to Cohen, et al., for example, relates to transformation of unicellular host organisms with "hybrid" viral or circular plasmid DNA which includes selected exogenous DNA sequences. The procedures of the Cohen, et al. patent first involve manufacture of a transformation vector by enzymatically cleaving viral or circular plasmid DNA to form linear DNA strands. Selected foreign ("exogenous" or "heterologous") DNA strands usually including sequences coding for desired product are prepared in linear form through use of similar enzymes. The linear viral or plasmid DNA is incubated with the foreign DNA in the presence of ligating enzymes capable of effecting a restoration process and "hybrid" vectors are formed which include the selected exogenous DNA segment "spliced" into the viral or circular DNA plasmid.

Transformation of compatible unicellular host organisms with the hybrid vector results in the formation of multiple copies of the exogenous DNA in the host cell population. In some instances, the desired result is simply the amplification of the foreign DNA and the "product" harvested is DNA. More frequently, the goal of transformation is the expression by the host cells of the exogenous DNA in the form of large scale synthesis of isolatable quantities of commercially significant protein or polypeptide fragments coded for by the foreign DNA. See also, e.g., U.S. Pat. Nos. 4,264,731 (to Shine), 4,273,875 (to Manis), 4,293,652 (to Cohen), and European Patent Application 093,619, published Nov. 9, 1983.

The development of specific DNA sequences for splicing into DNA vectors is accomplished by a variety of techniques, depending to a great deal on the degree of "foreignness" of the "donor" to the projected host and the size of the polypeptide to be expressed in the host. At the risk of over-simplification, it can be stated that three alternative principal methods can be employed: (1) the "isolation" of double-stranded DNA sequence from the genomic DNA of the donor; (2) the chemical manufacture of a DNA sequence providing a code for a polypeptide of interest; and (3) the in vitro synthesis of a double-stranded DNA sequence by enzymatic "reverse transcription" of mRNA isolated from donor cells. The last-mentioned methods which involve formation of a DNA "complement" of mRNA are generally referred to as "cDNA" methods.

Manufacture of DNA sequences is frequently the method of choice when the entire sequence of amino acid residues of the desired polypeptide product is known. DNA manufacturing procedures of co-owned, co-pending U.S. patent application Ser. No. 483,451, by Alton, et al., (filed Apr. 15, 1983 and corresponding to PCT US83/00605, published Nov. 24, 1983 as WO83/04053), for example, provide a superior means for accomplishing such highly desirable results as: providing for the presence of alternate codons commonly found in genes which are highly expressed in the host organism selected for expression (e.g., providing yeast or *E. coli* "preference" codons); avoiding the presence of untranslated "intron" sequences (commonly present in mammalian genomic DNA sequences and mRNA transcripts thereof) which are not readily processed by procaryotic host cells; avoiding expression of undesired "leader" polypeptide sequences commonly coded for by genomic DNA and cDNA sequences but frequently not readily cleaved from the polypeptide of interest by bacterial or yeast host cells; providing for ready insertion of the DNA in convenient expression vectors in association with desired promoter/regulator and terminator sequences; and providing for ready construction of genes coding for polypeptide fragments and analogs of the desired polypeptides.

When the entire sequence of amino acid residues of the desired polypeptide is not known, direct manufacture of DNA sequences is not possible and isolation of DNA sequences coding for the polypeptide by a cDNA method becomes the method of choice despite the potential drawbacks in ease of assembly of expression vectors capable of providing high levels of microbial expression referred to above. Among the standard procedures for isolating cDNA sequences of interest is the preparation of plasmid-borne cDNA "libraries" derived from reverse transcription of mRNA abundant in donor cells selected as responsible for high level expression of genes (e.g., libraries of cDNA derived from pituitary cells which express relatively large quantities of growth hormone products). Where substantial portions of the polypeptide's amino acid sequence are known, labelled, single-stranded DNA probe sequences duplicating a sequence putatively present in the "target" cDNA may be employed in DNA/DNA hybridization procedures carried out on cloned copies of the cDNA which have been denatured to single stranded form. [See, generally, the disclosure and discussions of the art provided in U.S. Pat. No. 4,394,443 to Weissman, et al. and the recent demonstrations of the use of long oligonucleotide hybridization probes reported in Wallace, et al., *Nuc. Acids Res.,* 6, pp. 3543–3557 (1979), and Reyes, et al., *P.N.A.S.* (*U.S.A.*), 79, pp. 3270–3274 (1982), and Jaye, et al., *Nuc. Acids Res.,* 11, pp. 2325–2335 (1983). See also, U.S. Pat. No. 4,358,535 to Falkow, et al., relating to DNA/DNA hybridization procedures in effecting diagnosis; published European Patent Application Nos. 0070685 and 0070687 relating to light-emitting labels on single stranded polynucleotide probes; Davis, et al., "A Manual for Genetic Engineering, Advanced Bacterial Genetics", Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1980) at pp. 55–58 and 174–176, relating to colony and plaque hybridization techniques; and, New England Nuclear (Boston, Mass.) brochures for "Gene Screen" Hybridization Transfer Membrane materials providing instruction manuals for the transfer and hybridization of DNA and RNA, Catalog No. NEF-972.]

Among the more signficant recent advances in hybridization procedures for the screening of recombinant clones is the use of labelled mixed synthetic oligonucleotide probes, each of which is potentially the complete complement of a specific DNA sequence in the hybridization sample including a heterogenous mixture of single stranded DNAs or RNAs. These procedures are acknowledged to be especially useful in the detection of cDNA clones derived from sources which provide extremely low amounts of mRNA sequences for the polypeptide of interest. Briefly put, use of stringent hybridization conditions directed toward avoidance of non-specific binding can allow, e.g., for the autoradiographic visualization of a specific cDNA clone upon the event of hybridization of the target DNA to that single probe within the mixture which is its complete complement. See generally, Wallace, et al., *Nuc. Acids Res.,* 9, pp. 879–897 (1981); Suggs, et al. *P.N.A.S.* (*U.S.A.*), 78, pp. 6613–6617 (1981); Choo, et al., *Nature,* 299, pp. 178–180 (1982); Kurachi, et al., *P.N.A.S.* (*U.S.A.*), 79, pp. 6461–6464 (1982); Ohkubo, et al., *P.N.A.S.* (*U.S.A.*), 80, pp. 2196–2200 (1983); and Kornblihtt, et al. *P.N.A.S.* (*U.S.A.*), 80, pp. 3218–3222 (1983). In general, the mixed probe procedures of Wallace, et al. (1981), supra, have been expanded upon by various workers to the point where reliable results have reportedly been obtained in a cDNA clone isolation using a 32 member mixed "pool" of 16-base-long (16-mer) oligonucleotide probes of uniformly, varying DNA sequences together with a single 11-mer to effect a two-site "positive" confirmation of the presence of cDNA of interest. See, Singer-Sam, et al., *P.N.A.S.* (*U.S.A.*), 80, pp. 802–806 (1983).

The use of genomic DNA isolates is the least common of the three above-noted methods for developing specific DNA sequences for use in recombinant procedures. This is especially true in the area of recombinant procedures directed to securing microbial expression of mammalian polypeptides and is due, principally to the complexity of mammalian genomic DNA. Thus, while reliable procedures exist for developing phage-borne libraries of genomic DNA of human and other mammalian species origins [See, e.g., Lawn, et al. *Cell,* 15, pp. 1157–1174 (1978) relating to procedures for generating a human genomic library commonly referred to as the "Maniatis Library"; Karn, et al., *P.N.A.S.* (*U.S.A.*), 77, pp. 5172–5176 (1980) relating to a human genomic library based on alternative restriction endonuclease fragmentation procedure; and Blattner, et al., *Science,* 196, pp. 161–169 (1977) describing construction of a bovine genomic library] there have been relatively few successful attempts at use of hybridization procedures in isolating genomic DNA in the absence of extensive foreknowledge of amino acid or DNA sequences. As one example, Fiddes, et al., *J. Mol. and App. Genetics,* 1, pp. 3–18 (1981) report the successful isolation of a gene coding for the alpha subunit of human pituitary glycoprotein hormones from the Maniatis Library through use of a "full length" probe including a complete 621 base pair fragment of a previously-isolated cDNA sequence for the alpha sub-

unit. As another example, Das, et al., *P.N.A.S.* (*U.S.A.*), 80, pp. 1531–1535 (1983) report isolation of human genomic clones for human HLA-DR using a 175 base pair synthetic oligonucleotide. Finally, Anderson, et al., *P.N.A.S.* (*U.S.A.*), 80, pp. 6838–6842 (1983) report the isolation of genomic clone for bovine pancreatic trypsin inhibitor (BPTI) using a single probe 86 base pairs in length and constructed according to the known amino acid sequence of BPTI. The authors note a determination of poor prospects for isolating mRNA suitable for synthesis of a cDNA library due to apparent low levels of mRNA in initially targeted parotid gland and lung tissue sources and then address the prospects of success in probing a genomic library using a mixture of labelled probes, stating: "More generally, mixed-sequence oligodeoxynucleotide probes have been used to isolate protein genes of unknown sequence from cDNA libraries. Such probes are typically mixtures of 8–32 oligonucleotides, 14–17 nucleotides in length, representing every possible codon combination for a small stretch (5–6 residues) of amino acid sequence. Under stringent hybridization conditions that discriminate against incorrectly base-paired probes, these mixtures are capable of locating specific gene sequences in clone libraries of low-to-moderate complexity. Nevertheless, because of their short length and heterogeneity, mixed probes often lack the specificity required for probing sequences as complex as a mammalian genome. This makes such a method impractical for the isolation of mammalian protein genes when the corresponding mRNAs are unavailable." (Citations omitted).

There thus continues to exist a need in the art for improved methods for effecting.the rapid and efficient isolation of cDNA clones in instances where little is known of the amino acid sequence of the polypeptide coded for and where "enriched" tissue sources of mRNA are not readily available for use in constructing cDNA libraries. Such improved methods would be especially useful if they were applicable to isolating mammalian genomic clones where sparse information is available concerning amino acid sequences of the polypeptide coded for by the gene sought.

B. Erythropoietin As A Polypeptide Of Interest

Erythropoiesis, the production of red blood cells, occurs continuously throughout the human life span to offset cell destruction. Erythropoiesis is a very precisely controlled physiological mechanism enabling sufficient numbers of red blood cells to be available in the blood for proper tissue oxygenation, but not so many that the cells would impede circulation. The formation of red blood cells occurs in the bone marrow and is under the control of the hormone, erythropoietin.

Erythropoietin, an acidic glycoprotein of approximately 34,000 dalton molecular weight, may occur in three forms: α, β and asialo. The α and β forms differ slightly in carbohydrate components, but have the same potency, biological activity and molecular weight. The asialo form is an α or β form with the terminal carbohydrate (sialic acid) removed. Erythropoietin is present in very low concentrations in plasma when the body is in a healthy state wherein tissues receive sufficient oxygenation from the existing number of erythrocytes. This normal low concentration is enough to stimulate replacement of red blood cells which are lost normally through aging.

The amount of erythropoietin in the circulation is increased under conditions of hypoxia when oxygen transport by blood cells in the circulation is reduced. Hypoxia may be caused by loss of large amounts of blood through hemorrhage, destruction of red blood cells by over-exposure to radiation, reduction in oxygen intake due to high altitudes or prolonged unconsciousness, or various forms of anemia. In response to tissues undergoing hypoxic stress, erythropoietin will increase red blood cell production by stimulating the conversion of primitive precursor cells in the bone marrow into proerythroblasts which subsequently mature, synthesize hemoglobin and are released into the circulation as red blood cells. When the number of red blood cells in circulation is greater than needed for normal tissue oxygen requirements, erythropoietin in circulation is decreased.

See generally, Testa, et al., *Exp. Hematol.,* 8(Supp. 8), 144–152 (1980); Tong, et al., *J. Biol. Chem.,* 256(24), 12666–12672 (1981); Goldwasser, *J. Cell. Physiol.,* 110 (Supp. 1), 133–135 (1982); Finch, *Blood,* 60(6), 1241–1246 (1982); Sytowski, et al., *Exp. Hematol.,* 8(Supp 8), 52–64 (1980): Naughton, *Ann. Clin. Lab. Sci.,* 13(5), 432–438 (1983); Weiss, et al., *Am. J. Vet. Res.,* 44(10),1832–1835 (1983); Lappin, et al., *Exp. Hematol.,* 11(7), 661–666 (1983); Baciu, et al., *Ann. N.Y. Acad. Sci.,* 414, 66–72 (1983); Murphy, et al., *Acta. Haematologica Japonica,* 46(7), 1380–1396 (1983); Dessypris, et al., *Brit. J. Haematol.,* 56, 295–306 (1984); and, Emmanouel, et al., *Am. J. Physiol.,* 247 (1 Pt 2), F168–76 (1984).

Because erythropoietin is essential in the process of red blood cell formation, the hormone has potential useful application in both the diagnosis and the treatment of blood disorders characterized by low or defective red blood cell production. See, generally, Pennathur-Das, et al., *Blood,* 63(5), 1168–71 (1984) and Haddy, *Am. Jour. Ped. Hematol./Oncol.,* 4, 191–196, (1982) relating to erythropoietin in possible therapies for sickle cell disease, and Eschbach, et al. *J. Clin. Invest.,* 74(2), pp. 434–441, (1984), describing a therapeutic regimen for uremic sheep based on in vivo response to erythropoietin-rich plasma infusions and proposing a dosage of 10 U EPO/kg per day for 15–40 days as corrective of anemia of the type associated with chronic renal failure. See also, Krane, *Henry Ford Hosp. Med. J.,* 31(3), 177–181 (1983).

It has recently been estimated that the availability of erythropoietin in quantity would allow for treatment each year of anemias of 1,600,000 persons in the United States alone. See, e.g., Morrison, "Bioprocessing in Space—an Overview", pp. 557–571 in The World Biotech Report 1984, Volume 2:USA, (Online Publications, New York, N.Y. 1984). Recent studies have provided a basis for projection of efficacy of erythropoietin therapy in a variety of disease states, disorders and states of hematologic irregularity: Vedovato, et al., *Acta. Haematol,* 71, 211–213 (1984) (beta-thalassemia); Vichinsky, et al., *J. Pediatr.,* 105(1), 15–21 (1984) (cystic fibrosis); Cotes, et al., *Brit. J. Obstet. Gyneacol.,* 90(4), 304–311 (1983) (pregnancy, menstrual disorders); Haga, et al., *Acta. Pediatr. Scand.,* 72, 827–831 (1983) (early anemia of prematurity); Claus-Walker, et al., *Arch. Phys. Med. Rehabil.,* 65, 370–374 (1984) (spinal cord injury); Dunn, et al., *Eur. J. Appl. Physiol.,* 52, 178–182 (1984) (space flight); Miller, et al., *Brit. J. Haematol.,* 52, 545–590 (1982) (acute blood loss); Udupa, et al., *J. Lab. Clin. Med.,* 103(4), 574–580 and 581–588 (1984); and Lipschitz, et al., *Blood,* 63(3), 502–509 (1983) (aging); and Dainiak, et al., *Cancer,* 51(6), 1101–1106 (1983) and Schwartz, et al., *Otolaryngol.,* 109, 269–272 (1983) (various neoplastic disease states accompanied by abnormal erythropoiesis).

Prior attempts to obtain erythropoietin in good yield from plasma or urine have proven relatively unsuccessful. Complicated and sophisticated laboratory techniques are necessary and generally result in the collection of very small amounts of impure and unstable extracts containing erythropoietin.